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Part I

THE CHEMISTRY AND TECHNOLOGY OF THE SOYBEAN AND ITS DERIVED PRODUCTS

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By

Klare S. Markley and Warren H. Goss

U. S. Regional Soybean Industrial Products Laboratory

Urbana, Illinois

Part I

Chemical Composition and Properties of Constituent and Derived Products

By Klare S. Markley

Effective July 1, 1942, this Laboratory was transferred by Act of Congress to the
NORTHERN REGIONAL RESEARCH LABORATORY
PEORIA, ILLINOIS

United States Department of Agriculture
Bureau of Agricultural Chemistry and Engineering

1942

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INTRODUCTION

The soybean, Soja max (L.) Piper (349), 1/ also referred to by botanists as Soja hispida (Moench) and Glycine hispida (Moench) Maxim., is one of the oldest crops cultivated by man. Its early history is lost in antiquity. According to Morse and Cartter (287), the plant was extensively cultivated in China long before written records were kept. According to these authors, the first record of the plant is contained in a materia medica written by Emperor Sheng Nung in 2838 B. C. Throughout the history of China and down to the present day the soybean has been the most important cultivated legume, and probably the most important crop, to the existence of the Chinese people and to the Chinese civilization.

From China the soybean has spread over a considerable portion of the world. The principal soybean producing countries are China, Manchuria, United States of America, Chosen, Japan, and Netherlands India in the order named. In the last few years increasing quantities of soybeans have been grown in Europe especially in Germany, the Balkan States, and the Union of Soviet Socialist Republics. England, South Africa, British East Africa, Algeria, Egypt, New South Wales, and New Zealand, have experimented with soybeans, but commercial production has been negligible. The trend of soybean production in various countries is shown in Table 1, page 3, which is reproduced from Foreign Crops and Markets (440).

Although China usually produces more soybeans than any other country and approximately as much as the two next largest producers, Manchuria and the United States, it is not a factor in world trade as practically the total production is domestically consumed. In addition to domestic production, China normally takes important quantities of soybeans and soybean products from Manchuria. The latter country is the most important factor in the world soybean trade, although in 1939 the United States exported 10.5 million bushels to European oil centers, principally the Netherlands and Scandinavian countries. Japan has been one of the largest importers of soybeans from Manchuria, but these imports have been decreasing. Germany's imports of soybean have increased rapidly in recent years, reaching approximately 800,000 metric tons in 1938. Most of these imports came from Manchuria, but the United States, Rumania, and other Balkan States contributed to the total. The world production for 1937 has been estimated at 12,454,000 metric tons or approximately 457,500,000 United States bushels. Data covering imports and exports of soybeans and soybean oil for all principal producing and consuming countries for the period of 1930 to 1937 are contained in the Report of the Imperial Economic Committee (193) to which the reader is referred for detailed information on this subject.

In the Orient, especially in China and Japan, soybeans are grown principally for the seed which has served for centuries as an important source of food. For this purpose the beans are utilized in the preparation of numerous fresh, fermented, and dried products. Most familiar to Occidentals is the

1/ Numbers in parentheses refer to Literature Cited.

Table 1. Soybeans: Production in specified countries, 1935 to 1941

Country	1935	1936	1937	1938	1939	1940	1941 a/
	1,000 bushels	1,000 bushels	1,000 bushels	1,000 bushels	1,000 bushels	1,000 bushels	1,000 bushels
China b/	184,415	215,728	213,189	207,600	203,900	216,800	c/
Manchuria ...	141,793	152,375	159,907	157,445	144,952	140,617	c/
United States	44,378	29,983	45,272	62,729	91,272	77,374	106,712
Chosen	20,738	17,937	20,205	18,333	c/	c/	c/
Japan	10,719	12,485	13,473	c/	c/	c/	c/
Taiwan	139	162	158	146	c/	c/	c/
Netherlands Indies..	7,448	9,090	9,880	10,567	c/	c/	c/
Rumania.....	424	1,367	2,584	1,803	3,532	d/3,600	c/
Bulgaria.....	c/	179	419	246	613	1,415	c/
Yugoslavia ..	36	22	54	140	103	294	c/
Hungary.....	c/	c/	c/	c/	125	194	c/
Total e/...	410,500	439,700	466,000	473,000	477,500	483,500	c/

Compiled from official sources and unofficial estimates.

a/ Preliminary.

b/ Excluding Kwangsi Province for 1935 to 1937, which normally produces about 2,500,000 bushels.

c/ Not available.

d/ Assuming that Bessarabia produces 80 percent of the total.

e/ Includes a small amount for other minor-producing countries but excludes the Soviet Union.

fermented product known as shoyu or soy sauce which is but one of the numerous derivatives of this versatile seed. Besides soy sauce, the beans are used in the form of flour, vegetable milk (fresh, condensed, and dried), curd or tofu from which a variety of products are prepared, cheese, and the important fermented product known as miso. Large quantities of beans are also crushed for oil which is used for food and numerous industrial purposes, and the resulting cake or meal is used chiefly as a fertilizer and as feed for animals. The roots are often dug and dried for fuel, especially in Manchuria, and the leaves, stalks, pods, and other refuse are used for feeding cattle. The use of soybean stalks as a source of cellulose pulp (249, 18) has been proposed and a plant capable of producing 15,000 tons of pulp annually was erected at Kaiyan, Manchuria, in 1939. A Japanese patent (283) covering the use of soybean refuse mixed with powdered coal and lime and pressed into briquets for fuel has been granted.

In contrast to the food uses in the Orient, European countries have for many years imported considerable quantities of soybeans from Manchuria for the production of oil and meal. In the United States the soybean has been used primarily for forage purposes, being either preserved extensively as hay or silage or cut and fed in the green state. It is also pastured extensively with hogs and sheep and used to some extent as a green manure or cover crop. More recently, soybeans have been grown extensively in the United States for the production of oil. During the last 15 years both the total acreage sown to soybeans and the acreage harvested for commercial beans, have greatly increased as is evident from Table 2, page 5.

"The marked increase in the production of soybeans in the Corn Belt has been conditioned by several factors. These include the ability of the soybean plant to withstand drought, its relative freedom from pest hazards, its adaptability to crop rotations, the possibility of harvesting the beans with the small combine, and the fact that soybeans provide an additional source of cash income for many farmers. The necessity for finding a more profitable crop than oats, and in some cases corn, has been important in bringing about increased soybean production. In the South, where cotton acreage has been reduced considerably in recent years, soybeans, harvested mostly for hay, forage, and as a green-manure crop, also have been useful for replacement purposes." (444)

The marked increase in the production of soybeans in the United States has led in the last few years to the development of numerous food, feed, and industrial uses for both the meal and the oil. There are at present over 100 oil mills 2/ processing soybeans and several times that number of concerns are engaged in the manufacture of soybean flour and other food products and in the manufacture of various industrial products. Table 3 prepared by Mr. W. J. Morse of the United States Department of Agriculture shows the diversity of uses to which the different products of the soybean are put.

2/ See list of oil mills in Part II.

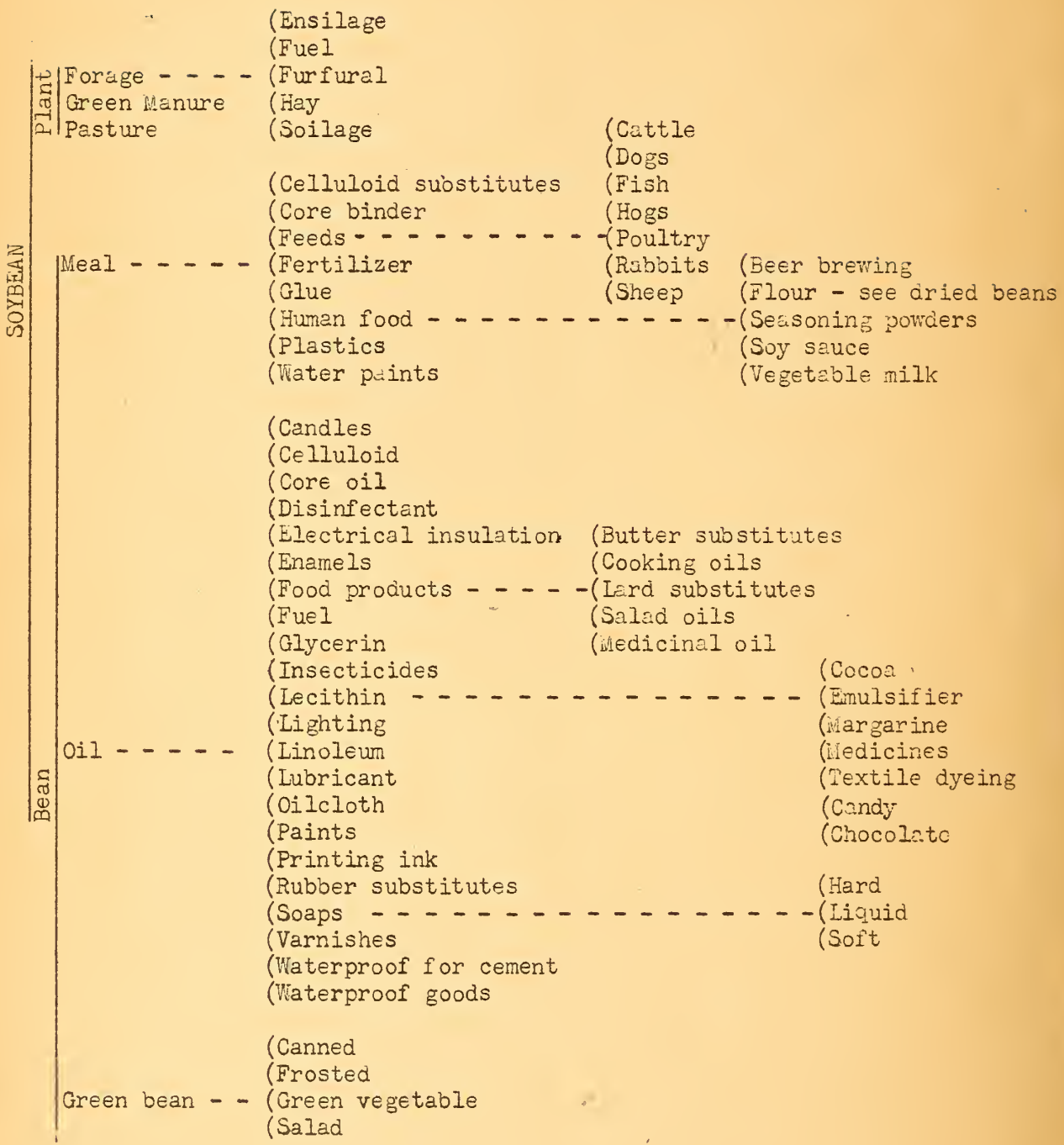
Table 2. Soybeans: Acreage, yield, and production, United States 1924-1940 1/

Year	Acreage grown alone for all purposes	Acreage harvested for beans	Average yield per acre	Production
	1,000 acres	1,000 acres	bushels	1,000 bushels
1924	1,567	448	11.0	4,947
1925	1,539	415	11.7	4,875
1926	1,871	466	11.2	5,239
1927	2,057	568	12.2	6,938
1928	2,154	579	13.6	7,880
1929	2,400	708	13.3	9,398
1930	3,010	1,008	13.4	13,471
1931	3,738	1,104	15.2	16,733
1932	3,595	977	15.3	14,975
1933	3,365	997	13.2	13,147
1934	5,572	1,539	15.0	23,095
1935	6,640	2,697	16.5	44,378
1936	5,811	2,132	14.1	29,983
1937	6,171	2,549	17.8	45,272
1938	7,262	3,105	20.2	62,729
1939	9,509	4,417	20.7	91,272
1940	10,528	4,961	16.1	77,374
1941 2/	9,996	5,855	18.2	106,712

1/ Agricultural Statistics. U. S. Department of Agriculture, p. 299 (1941).

2/ Agricultural Marketing Service. General Crop Report.

Table 3. Soybean Utilization



	(Baked		(Cattle		(Baked products
	(Boiled		(Hogs		(Breakfast foods
	(Breakfast foods		(Poultry		(Candies
	(Feeds		(Sheep		(Chocolate
Dried bean	(Flour				(Diabetic foods
	(Roasted			(Candied	(Health drinks
	(Soy sauce		(Paints	(Coffee substitute	(Ice cream cones
	(Sprouts		(Paper size	(Salted	(Ice cream powder
	(Vegetable milk -	(Casein - -	(Textile dressing		(Infant foods
		(Condensed	(Waterproofing	(Canned	(Macaroni products
		(Curd		(Dried	(Meat products - filler
		(Foods		(Fermented	
		(Powder		(Fresh	
				(Smoked	

The soybean is an annual summer legume requiring from 75 to 200 or more days to reach maturity. The plant which grows to a height of from 2 to more than 6 feet, though more usually from 2 to 3-1/2 feet, has rather woody and branched stems (fig. 1).

The stems, leaves, and seed pods are covered with short reddish-brown or gray hairs. The flowers are small and inconspicuous, either white or purple, and clustered in the axils of the leaves. The pods are from 1 to 2-1/2 inches long and contain 2 to 4 seeds. When mature, the seeds may be yellow, green, brown, or black in color; and in shape, round, oval, or flattened like the lima bean.

The varieties of soybeans are very numerous and many hundreds of types and strains are known. They may be divided into three general groups with respect to their uses, namely, vegetable varieties, hay varieties, and grain or industrial varieties. The unusually large number of known varieties and strains of soybeans appears to be the result of the peculiar sensitivity of the plant to changes in soil and climate. The recognition of the sensitivity of the soybean to environment has been the basis of much of the breeding and adaptation studies which have been carried on in various countires of the world, particularly in the United States, Japan, Manchuria, China, India, Chosen, Germany, the Union of Soviet Socialist Republics, and other European countries. The chief objective of these studies has been to increase the yield of beans under local conditions; but, more recently, similar work has been conducted with a view of increasing the quality of beans, especially for industrial utilization.

The factors or characters which are included in the term quality as applied to the industrial uses of soybeans are principally oil content, iodine number of the oil, phosphatide content, and protein content. In response to the demand by oil mills for varieties of high oil content, breeding and selection from foreign introductions into the United States have led to the development of yellow-seeded varieties such as Illini, Dunfield, Mukden, Mandell, Scioto, Mansoy, Manchu, Mamredo, Delsta, Mandarin, Seneca, and many others which are particularly adapted to a given agricultural region.

The literature pertaining to the effect of variety, climate, soil type, and maturity on the yield and quality of protein and oil is very extensive and has been reported from every country in which the soybean has been grown. A similarly extensive literature pertaining to the cultural practices, including harvesting and handling of the mature seed intended for commercial processing, is readily available.

COMPOSITION AND PROPERTIES

From the chemical point of view most seeds are extremely complex, containing as they do all the elements and compounds necessary to reproduce and nourish the new plant until it can form the necessary system of roots and leaves to obtain from the soil and atmosphere the materials essential for growth and maturation. The nature and chemical composition of some of the numerous products stored by seeds are known and their functions in the reproduction of the plant are understood. However, many substances which are present in seeds have not been completely characterized, chemically, and their probable functions provide food only for speculation.

In foliage

Mature

Figure 1.- The soybean plant, in foliage and mature.

To this general statement the soybean is no exception and despite the considerable study which has been devoted to the composition of the soybean seed, it may be said that only its grossest aspects are known and much is yet to be learned of the finer and less obvious details of its chemical structure. It is well known, for example, that the soybean seed is comprised principally of protein, oil, and carbohydrates, and mineral constituents. It is also known that the protein is made up of a number of amino acids; that the oil contains a variety of mixed fatty acid glycerides and phosphatides as well as unsaponifiable materials such as sterols, ketones, and hydrocarbons; that the carbohydrates are present in the form of sugars, cellulose, and various glycosides; and that the mineral constituents consist of potassium, calcium, phosphorus, and similar elements. Moreover, the soybean seed contains many minor constituents such as pigments, vitamins, enzymes, and antioxidant bodies, many of which have never been isolated in pure form. Little is known regarding their structure, function, and use. Still other substances are undoubtedly present in the soybean seed, but only a guess may be hazarded as to their identity.

Not only are the chemical constituents of the soybean numerous and complex, but their relative proportions often vary markedly as a result of differences in variety, state of maturity, soil, fertilizer treatment, cultural and climatic conditions, as well as other environmental factors. The extant literature pertaining to the effect of the above-mentioned factors on the chemical composition of the soybean is voluminous and has been reported from every country in which the soybean has been grown.

Typical of these investigations may be mentioned the work of Arasimovich, Artemieva, and Pavlova (19) who noted that the protein content decreased when soybeans were grown under irrigation. Cartter and Milner (75) found the yield of protein and oil varied with different varieties of soybeans when grown in the same locality, and also when the same variety was grown in different localities. Hall 3/ examined a large number of soybeans, representing shipments of the 1935 crop received at various United States terminal markets, and found that the protein content varied from 32.8 to 45.0 percent, and the oil content from 18.5 to 22.1 percent, calculated on an oven-dry basis. McClelland (257) examined 160 varieties of soybeans and found the oil content to vary from 13 to 23 percent. O'Kelly and Gieger (331) observed marked variations in the content of oil, protein, nitrogen free extract, ash, and crude fiber of the same variety of soybeans as a result of differences of maturity, crop year, and soundness of the seed, as well as similar marked differences when the same variety was compared for different crop years.

Piper and Morse (349) state that the principal varieties in the United States vary from 34.1 to 46.9 percent in protein content.

Tsukunaga and Nishino (439) followed the changes in composition of soybeans from 53 to 54 days after blooming until time of normal maturity and found the nitrogen free extract, crude fiber and ash content decreased with increased maturation while the protein and oil content increased. Sessous (390) reported a variation in protein content from 31.7 to 41.9 percent and in the oil content from 16.5 to 21.3 percent for all varieties grown at

3/ Hall, Wallace L., Some analyses of commercial soybeans: U. S. Bureau of Agricultural Economics, 5 pp. 1937 (Mimeographed).

Geissen from 1929 to 1936. Similar variations in the protein and oil contents were observed by Webster and Kiltz (450) for Oklahoma grown soybeans and by Shutt (397) for seed grown in Canada. Smirnova and Lavrova (399) examined a number of varieties of soybeans to determine the effect of geographic location on their protein, oil, ash, and enzyme content. Viljoen (441) examined a number of varieties of soybeans with regard to the effect of seasonal conditions and soil types and concluded that variations in the fertility of the soil, as ordinarily encountered in farm practice in South Africa, had little or no effect on the composition of the bean, but variations in climatic conditions had a very pronounced effect. According to the observations of Viljoen, for every degree Fahrenheit rise or fall in the minimum temperature, a corresponding increase or decrease of 0.44 percent in the oil content may be expected. No correlation was found between the maximum temperature and percentage of oil and protein, or between rainfall and these two characters. Woodworth (470) found the oil content of Illini soybeans ranged from 18.95 to 21.02 percent when grown in different parts of Illinois.

Bailey, Capen, and LeClerc (25) summarized the results of hundreds of analyses of soybeans as indicated in Table 4. These authors state that soybeans

Table 4. Chemical Composition of Soybeans

	Minimum	Maximum	Average
	Percent	Percent	Percent
Moisture	5.02	9.42	8.0
Ash	3.30	6.35	4.6
Fat	13.50	24.20	18.0
Fiber	2.84	6.27	3.5
Protein	29.60	50.30	40.0
Pentosan	3.77	5.45	4.4
Sugars	5.65	9.46	7.0
Starch-like substances by diastase	4.65	8.97	5.6

consist of 6 to 8 percent of hull, 90 to 92 percent of cotyledons, and 1.5 to 2 percent of germ having the composition given in Table 5.

Table 5. Composition of the Component Parts of Soybeans

	Proportion of the seed	Moisture	Protein	Carbo-hydrates	Fat	Ash
	Percent	Percent	Percent	Percent	Percent	Percent
Cotyledons	90	10.57	41.33	14.60	20.75	4.38
Germ	2	12.01	36.93	17.32	10.45	4.08
Seed coat	8	12.53	7.00	21.02	.60	3.83

MINERAL CONSTITUENTS

The ash content of soybeans varies considerably with the variety and the amount and availability of the mineral constituents of the soil, but five percent may be taken as a fair average for air-dry beans. (See Table 6.) They contain more calcium and phosphorus than any of the cereal grains, and few foods excel soybeans as a source of iron, four-fifths of which is biologically available according to Sherman, Elvehjem, and Hart (394).

Table 6. Mineral Content of Soybeans (air-dry basis).[1/]

	Percent
Ash	5.06
Potassium	1.91
Sodium	0.34
Calcium	0.21
Iron	0.0074
Copper	0.0012
Magnesium	0.22
Phosphorus	0.59
Sulfur	0.41
Chlorine	0.024
Manganese	0.0028
Zinc	0.0018
Aluminum	0.0007

1/ The figures for copper, manganese, and zinc are taken from Winton's Structure and Composition of Foods (466). Aluminum reported by Paul Meunier (273). The other figures are from Forbes, Beegle, and Mensching (122).

Hirano and Mikuno (184) have reported the presence of 15.896 mg. of copper per kilo of soybeans, 30 to 40 percent of which is found in the seed coat. In view of the fact that a few parts per million of copper salts (oleate) adversely affects the stability or keeping quality of most refined oils, it would be of interest to know how much of the total copper contained in soybeans finds its way into the refined oil. Spectrographic analysis has shown copper to be present in refined soybean oil, but the amount and manner of its combination are unknown.

At one time it was presumed that the phosphorus in soybeans was present solely in the form of phosphatides, but according to Earle and Milner (105) only a relatively small portion of the total phosphorus is actually present in this form as is evident from the results shown in Table 7. In fact,

Table 7. Distribution of Phosphorus in Dunfield Soybeans Containing 6.02 mg. Phosphorus per Gram of Whole Bean

Sample No.	Phosphatide Phosphorus	Inorganic Phosphorus	Phytin Phosphorus	Phosphorus in Residue	Total accounted for
	Mg.	Mg.	Mg.	Mg.	Mg.
1	.80	.27	4.45	.24	5.76
2	.77	.26	4.25	.33	5.61
3	.91*	-	4.44	.36	5.71
4	.91*	-	4.29	.37	5.57
5	.74	.26	4.33	.33	5.66
6	.74	.28	4.26	.32	5.60

*Also includes inorganic phosphorus.

approximately 75 to 80 percent of the phosphorus contained in the beans is present in the form of phytin.

The plant phytins are the calcium, magnesium, and potassium salts of phytic acid, mesoinositol hexaphosphoric acid, and the extent of their presence in the soybean is normally sufficient to account for practically all of the calcium and magnesium. McCance and Widdowson (256) have shown that 20 to 60 percent of ingested phytin is excreted unchanged, which may, therefore, account for the apparent calcium deficiency of soybeans which has been noted by various investigators. Crystalline

calcium oxalate has been observed to occur in the cells of the seed and has been known to precipitate from water extracts of soybean protein.

PROTEIN AND OTHER NITROGENOUS CONSTITUENTS

Meissl and Böcker (270) appear to have been among the earliest workers to make a systematic study of the chemical components of the soybean seed. Applying the procedure outlined by Ritthausen (373) for the fractional separation of the proteins of oilseeds, these authors extracted ground soybeans with water, dilute acids and alkalies, salts, etc., and examined the various extracts thus obtained. Based on the results of these examinations Meissl and Böcker concluded that the soybean contained 30 percent soluble "casein," 0.5 percent "albumin," 7 percent insoluble "casein," no gluten, and only small amounts of amido compounds. Of the protein soluble in dilute alkali they found 86.3 percent to be "casein" and 1.7 percent to be "albumin."

Little additional information concerning the nature of the proteins present in the soybean was forthcoming until the appearance of the classical work of Osborne and his co-workers. Osborne and Campbell (343) investigated the proteins derived from soybeans and proposed the name glycinin for the globulin which they considered to represent 80 to 90 percent of the total crude protein. A second and more soluble globulin similar to phaseolin was isolated, and an albumin-like proteid which they designated as legumelin was estimated to make up 1.5 percent of the total protein fraction. Osborne and Clapp (344) in their analyses of glycinin, the principal protein of the soybeans, found the content of amino acids was similar to the values reported for casein, the principal protein of milk.

Tadokoro and Yoshimura (419) fractionated the proteins present in soybeans by means of water, 10 percent sodium chloride solution, and 0.2 percent sodium hydroxide solution and obtained a number of products which they designated as glycinin, legumelin, and glutelin. They determined the various chemical and physical properties of the protein fractions including the ash, phosphorus, and nitrogen content; the specific rotatory power, hydrochloric acid combining power, and the nitrogen distribution.

Jones and Csonka (212) obtained five protein fractions from soybeans by fractional precipitation at definite concentrations of ammonium sulfate within a range of 33 to 70 percent of saturation. In order to determine which fraction represented glycinin, a salt extract of soybean meal was dialyzed. From the protein fraction which precipitated, two globulins were separated. One was precipitated from a 10 percent sodium chloride solution by ammonium sulfate at 55 percent of saturation and did not coagulate even at boiling temperatures. This fraction was called glycinin since its properties agreed with those given by Osborne and Campbell for glycinin.

Csonka and Jones (90) analyzed the chief protein, glycinin, from seeds of several varieties of soybeans and found it to differ considerably in amino content with greatest variations occurring in the content of cystine, a low value of 0.74 percent for the Illini variety and a high value of 1.45

percent for the Manchu variety. Csonka and Jones (91) were the first investigators to report on the amino acid distribution contained in the whole (nonprotein extracted) defatted soybean meal. Again cystine was found to be the most variable amino acid of the varieties tested. Values ranged from 0.287 percent for the Illini up to 0.491 percent for the Herman variety. The chief protein, glycinin, of the soybean contains the following percentage of amino acids: Glycine 0.97, valine 0.68, leucine 8.45, proline 3.78, phenylalanine 3.86, aspartic acid 3.89, glutamic acid 19.46, tyrosine 1.86 to 4.55, arginine 5.12, histidine 1.39, lysine 2.71, tryptophane 1.94 to 2.84, cystine 0.74 to 1.45, and methionine 1.84.

Hibino (177) separated albumin, glycinin (globulin), and glutelin from soybeans and studied the products derived from them after hydrolysis under various conditions. Hamilton, Uyei, Baker, and Grindley (147) determined the nitrogen distribution in a sample of soybeans containing 6.79 percent of this element. The basic nitrogen amounting to 28.94 percent of the total was found to be present as ammonia nitrogen 9.38 percent, humin nitrogen 2.87 percent, arginine nitrogen 15.70 percent, cystine nitrogen 1.46 percent, histidine nitrogen 5.60 percent, and lysine nitrogen 6.18 percent. The monoamino nitrogen amounted to 48.28 percent, nonamino 2.43 percent, ether soluble 0.16 percent, alcohol soluble 0.58 percent, and nonprotein 5.55 percent of the total nitrogen. Sasaki (378) reported the nitrogen distribution of a sample of Korean soybeans as follows: Total nitrogen 7.03 percent, protein nitrogen 6.48 percent, nonprotein nitrogen 0.55 percent. From the water extract of five kilograms of soybeans Sasaki obtained adenine (picrate) 1.40 g., guanine 0.31 g., histidine (dichloride) 0.62 g., arginine (nitrate) 9.55 g., choline (gold salt) 25.35 g., trigonelline (hydrochloride) 0.33 g., and an unidentified base 0.30 g.

Tomiyama (432) found soybeans to contain cystine 1.05 percent, methionine 3.01 percent, and tryptophan 1.35 percent. Sasaki (379) hydrolyzed whole soybean protein with 25 percent sulfuric acid for 32 hours, removed the diamino acids with phosphotungstic acid and the dicarboxylic amino acids with alcoholic barium hydroxide. The monoamino acids were then fractionated with the results shown in Table 8. (See Table 8 on page 15).

Okano and Beppu (329) examined soybean oil foots (probably obtained in the alcohol extraction process) and found them to contain arginine, aspartic acid, glutamic acid, hydroxyglutamic acid, and an unknown amino acid lactone having the formula $C_7H_{19}O_7N_3$. The lactone was obtained in the form of colorless prisms melting at 245° and was found to contain 2 carboxyl and 2 amino groups.

ENZYMES

Enzymes are the biochemical catalysts of all living organisms. Chemically the enzymes are proteins or substances closely related to proteins and in physical aggregation they are colloidal as they occur in nature. The presence in the soybean of a number of enzymes has been detected and reported

Table 8. Results of Fractionation of Monoamino Acids of Soybean Protein

Amino acid	Form in which separated	Percent of wt. of protein	Percent of total N (16.6 percent N in protein)
Glycine	Picrate	0.23	0.26
Alanine	Copper salt	4.12	3.90
Valine	---	2.56	1.84
Leucine	---	10.02	6.45
Isoleucine	Copper salt	2.38	1.53
Proline	Picrate	3.94	2.86
Phenylalanine	Hydrochloride and benzoic acid	5.21	2.66
Tryosine	---	3.82	1.78
Aspartic acid	Copper salt	15.09	3.23
Glutamic acid	Hydrochloride	16.60	9.44
Oxyglutamic acid	---	13.20	6.87
Serine	---	not found	--
Hydroxyproline	---	not found	--

by various workers, but a comprehensive study of the nature and activity of the enzymic systems present in the different organs of the soybean still remains to be made.

Urease: Perhaps the most thoroughly investigated enzyme found in soybeans is urease which is characterized by its specific action on urea. It is found in varying amounts in all leguminous plants and was the first enzyme to be obtained in crystalline form.

Takeuchi (420) investigated the presence of urease in the soybean and later Annett (17) reported its presence in all the varieties of soybeans which he examined.

According to Onodera (337, 338) the urease of soybeans loses its activity on dialysis but it is restored again by the addition of a small amount of fresh urease, indicating that the fresh urease contains a coenzyme. Heat, acid, and alkali were found to effect the coenzyme but not the enzyme. In germination urease accumulates in the germs of soybeans in large proportion, but the free coenzyme is absent.

The most comprehensive investigation on the soybean urease was reported by Wester in a series of communications extending over approximately five years. In the course of these investigations Wester (452, 453, 454, 455, 456, 457, 458, 459) examined the effect of bacteria, germination, age, variety, and other factors in relation to the urease activity of soybeans. He determined the urease content of 48 varieties of soybeans and found that all of them, regardless of age, possessed strong ureolytic activity.

Based on the examination of a number of varieties of soybeans Dox (104) concluded that differences existed in the urease activity of soybeans but these differences appeared to bear no relationship to the germinating power or the protein content of the seed. Urease activity was observed in seeds which were almost completely devitalized.

Kennaway (224) described in detail a method for the estimation of urea in blood using soybeans as a source of urease. Recently Granick (133, 134) reported the results of the distribution and variation of urease in various organs of the soybean as a function of maturation. Sizer (398) investigated the kinetics of urea hydrolysis with urease from two commercial preparations of soybean flour and found that at all temperatures carbon dioxide liberation was a linear function of elapsed time. The activation energies were found to be identical whether unextracted soybean or the purified enzyme was used.

Amylase: The presence of amylase in the soybean which had been reported as early as 1886 by Stingl and Morawski (405), was confirmed by Street and Bailey (408).

Orestano and Zummo (340) subjected dry, ground soybeans to various elevated temperatures for periods of 0.5 hour and extracted them with physiological salt solution after which their amylolytic activity was determined. It was concluded that their results did not support the hypothesis of the existence of two different enzymes, one of which caused liquefaction and the other saccharification of starch.

Later Artom and Orestano (20) studied the rate of starch liquefaction as a function of the change of viscosity and found it to be proportional to the square root of the temperature and approximately proportional to the concentration of the enzyme. Equations for the course of liquefaction and saccharification reactions were devised. Still later Orestano (339) reported that the soybean contained only one amylase, probably β-amylase. Teller (423, 424), however, concluded that soybeans contained two types of sugar-forming diastase which have different activities in starch media of different pH and at different temperatures. One of these diastases is of the type characteristic of normal wheat flour and is designated as "reserve" diastase. It was found in the cotyledons of soybeans in all stages of maturity. The other type which predominates in the bran of germinating cereals and in those plant parts most closely associated with active vegetation is designated as "vegetative" diastase. It was found to be present in the hull of soybeans and also in the pods and leaves.

Newton and Naylor (318) developed methods for preparing amylase concentrates from soybeans and for the determination of the saccharogenic power of soybeans and soybean concentrates. The application of these and other methods to soybeans indicated that the concentrates contain principally β-amylase. In a subsequent publication Martin, Naylor, and Hixon (264) reported the results of an investigation of the action of β-amylase from soybeans on corn, wheat, rice, potato, and tapioca starches.

Katagiri and Mugibayasi (219) investigated the maltase, saccharifying, and liquefying enzymes of germinated and ungerminated mature and green soybeans. Maltase and the liquefying enzyme were found to be absent in both the mature and germinated seeds.

Lipase: Falk (118) investigated the action of lipase from soybeans using the same methods as had been previously used for the lipase from castor beans. Soybean lipase was found to act on triacetin in various substrates in a manner quite comparable to castor bean lipase. Heat and desiccation were likewise found to effect both lipases similarly.

Barton (28) studied the range and activity of lipase from soybeans and concluded that it was identical with lipase from castor beans but less intense in its activity owing to the smaller amount in the former seed. Smirnova and Lavrova (399) have reported that, although the lipase activity of soybeans varies but little with the variety, the peroxidase activity varies from 7.56 to 88.02 units, and the urease activity from 26.86 to 60.76 units. André and Hou (14, 15, 16) and Horovitz-Vlasova and coworkers (189) reported the presence of a lipoxidase in soybeans.

Protease and other enzymes: Street and Bailey (408) found evidence of the presence of a protease of the peptoclastic type, as well as of a peroxidase and a lipase, but negative results were obtained for the presence of sucrase and protease of the peptonizing type. Blagowestschenski and Melamed (38), however, found various proteolytic enzymes were present in the soybean.

Hibbard (176) reported an extensive study of the various enzyme systems, including catalase, peroxidase, invertase, amylase, and reductase, of the soybean plant especially in relation to photoperiodism.

Ito (197) reported the presence of an ascorbic acid oxidase in the soybean.

CARBOHYDRATES

Despite the nutritional, technological, and economic importance of the carbohydrates present in soybeans these substances have not been completely characterized, and even the total amount of them which are present in the seed has not been agreed upon. According to Piper and Morse (349) the total carbohydrates amount to 22 to 29 percent, but exactly what carbohydrates are included is not stated. Many investigators have reported data on the presence of nitrogen-free extract and crude fiber in soybeans but such determinations

shed little light on the specific nature or composition of these carbohydrate materials. For example, O'Kelly and Gieger (331) have reported data covering a large number of determinations of nitrogen-free extract which were found to vary from 17.73 to 30.18 percent for sound mature beans. Determinations of crude fiber gave values of 4.37 to 13.17 percent and similar variations have been reported by other workers.

The difficulty in obtaining satisfactory quantitative data may be appreciated when it is realized that the carbohydrate substances are present in the soybean in many different forms including celluloses, free sugars, pentosans, hexosans, glycosides, and probably other forms.

The cellulosic fraction appears to have received little attention aside from crude fiber determinations and no attempts appear to have been made to separate the various celluloses or cellulose complexes such as ligno-cellulose or cuto-cellulose if such be present.

The presence in soybean seeds of the tetrasaccharide, stachyose, $C_{24}H_{42}O_{21}$ was first demonstrated by Tanret (421) who showed by hydrolysis that the sugar was a polysaccharide composed of one molecule of levulose, one molecule of glucose, and two molecules of galactose. This sugar is obtained as a by-product in the alcohol extraction process for the production of soybean oil.

Street and Bailey (408) reported the presence of the following carbohydrates in the soybean: Galactan 4.86, pentosan 4.94, invert sugar 0.07, sucrose 3.31, raffinose 1.13, starch 0.50, cellulose 3.29, undertermined hemicelluloses 0.04, dextrin 3.14, total 21.28. Sato (380) reported the presence of 21.66 percent of carbohydrates, distributed as follows: Sucrose 5.90, stachyose 3.52, araban 3.80, galactan 4.62, crude fiber 3.82. Kraybill, Smith, and Walter (238) reported the isolation of crystalline sucrose from soybeans. Iwasa (198, 200, 201, 202) obtained a sirup as a by-product in the alcohol extraction of soybeans which, on examination, was found to contain water 17.43 percent, carbohydrates 70.09 percent, protein 5.20 percent, and fat 2.28 percent. The carbohydrate fraction contained glucose, fructose, galactose, rhamnose, arabinose, glucuronic acid, sucrose, and stachyose. The ratio of sucrose to stachyose was found to be 3.50 to 1.90.

Burrell and Wolfe (70) reported the variation in composition of 5 varieties of edible soybeans which were grown, harvested, and stored under comparable conditions. Appreciable differences were found in the content of the various carbohydrates especially reducing sugars, sucrose, raffinose, pentosans, and galactans. No effort was made to determine the amount of stachyose which was present.

Considerable difference of opinion exists concerning the presence of starch in soybeans. A number of investigators have reported its presence in amounts up to 3 percent, while an equal number of investigators have reported their inability to demonstrate its presence. The discrepencies may perhaps be reconciled by differences in maturity of the beans used in the various experiments. In any event, the amount of starch is quite small.

GLYCOSIDES

The glycosides present in soybean seeds comprise a heterogeneous group of complex carbohydrate substances. At least three distinct carbohydrate-containing classes of compounds 6/ have been isolated from the soybean. One of these groups of compounds is known as the saponins which consist of an aglycone of the trimethylnaphthalene or triterpenoid hydropicene series combined with one or more molecules os sugar; another group comprises the phytosterolins, the aglycone portion of which is represented by a sterol likewise combined with a sugar molecule; and the third group comprises the isoflavone glycosides or yellow pigments contained in the cells of the cotyledon. The aglycones of this last group consist of di- and tri-hydroxy isoflavones which are combined with glucose.

Although differing in the aglycone portions of the molecule and hence in the chemical classification generally assigned to these substances, it is nevertheless convenient to treat them as a group on the basis of the carbohydrates which they contain.

Saponins: The saponins are widely distributed in plants and are generally characterized by their solubility in dilute alcohol and in aqueous acids and alkalis, foaming or lathering properties, bitter taste, and in many cases by their haemolytic action.

In 1923 Muramatsu (300) isolated what he assumed to be a hydroxy acid which he named hispidic acid. The product melted at 224°, gave a hexa-acetyl derivative melting at 155°, and corresponded to the formula $C_{34}H_{68}O_{17}$. Later he isolated from soybeans a compound (301) having the formula $C_{50}H_{50}O_{20}$ which melted at 240° and which he also presumed to be a new hydroxy acid. In reality these compounds were a mixture of saponins as was shown by later workers. By neutralizing a 70 percent alcoholic solution of free saponin with sodium hydroxide, Sumiki (410, 411) isolated from soybeans the sodium salt of a saponin melting at 222-4° and corresponding to the formula, $C_{52}H_{80}O_{21}$. The haemolytic power of the saponin was found to be relatively weak compared to standard Merck saponin. Hydrylysis of the saponin yielded the equivalent of one molecule of sapogenin melting at 228-241° and corresponding to the formula $C_{32}H_{50}O_3$, together with one molecule each of glucose, rhamnose, arabinose, and an acid which was presumed to be mesoxalic acid.

Walz (445) isolated three saponins from soybeans which he designated as glycosides C, C_1, and C_2. The saponins were obtained as white, characteristic crystalline products melting at 272°, 225°, and 278-280°, respectively. Preparation C when tested with canine erythrocytes gave a Kofler haemolytic index (232) of 1:1600, 1:200, 1:51200 for pH values of 6.0, 8.5, and 10.0. Saponin C_1 gave an entirely negative reaction and C_2 was not examined for

6/ The term glycoside is now used as a general name for the group, irrespective of the sugar present; glucoside is the specific name used for those glycosides, the sugar constituent of which is known to be glucose.

its haemolytic action. After hydrolysis crystalline sapogenins melting at 235-6° and 243-5° were obtained from saponins C and C_1, respectively, and glucose was detected qualitatively.

Okano and Ohara (328) examined the alcohol extract of soybeans from which the oil was removed with the object of identifying the soluble glycosidic materials. The alcohol solution was treated with sodium chloride solution and the resulting precipitate separated by filtration and extracted with ether. The ether insoluble portion was repeatedly crystallized from 80 percent ethanol whereby a crystalline saponin was obtained which melted at 225-7° and corresponded to the formula $C_{48-50}H_{77-81}O_{18}$. On hydrolysis there was obtained a crystalline sapogenin corresponding to the formula $C_{30-32}H_{48-52}O_3$, glucuronic acid, galactose, and rhamnose.

From the alcoholic mother liquors of the crystalline saponin, a second and amorphous saponin was obtained which melted at 216-18° and corresponded to the formula $C_{49-51}H_{79-83}O_{19}$. Five isomeric sapogenins were obtained depending on the degree of hydrolysis of the original saponin. Neither of the original saponins exhibited any haemolytic power.

Hattori, Ogimura, and Okumura (168) isolated two saponins from soybeans which they designated as saponins I and II. Saponin I melted at 265° and gave on hydrolysis a sapogenin melting at 249°, while saponin II melted at 217-218° and gave a sapogenin melting at 241°.

Burrell and Walter (69) likewise isolated a saponin from soybeans, which melted at 220-225°, and after prolonged hydrolysis, obtained and identified the sugars as galactose and probably rhamnose. Although the saponins isolated by all these workers gave different aglycones, Burrell and Walter assumed that their product and those of Sumiki, Walz, and Okano and Ohara were probably identical. In 1937 Miyasaka (284) demonstrated that apparently homogenous saponin derived from soybeans could yield at least three different sapogenins depending on the conditions of hydrolysis, and about the same time Nozoye and Katsura (323) reported the isolation of four different sapogenins from soybean saponin. Miyasaka assigned formulas corresponding to C_{29}, C_{27}, and C_{23} to his products, while Nozoye and Katsura assigned C_{30} formulas to all four of theirs. The properties of the seven sapogenins differ but little despite the apparent differences in composition.

Ochiai, Tsuda, and Kitagawa (324) in a comprehensive investigation of the saponins of soybeans separated the crude product in the form of a calcium salt from an alcohol extract of soybeans which had been freed of oil and phosphatides. The crude calcium salt contained in addition to saponins the phenolic glycoside, genistin. (See isoflavone glycosides). The purified crystalline saponin obtained from the calcium salt melted at 222°. After hydrolysis with acid and subsequent chromatographic adsorption, four sapogenins were obtained melting at 311°, 259°, 239°, and 298°, having the formulas $C_{30}H_{50}O_4$, $C_{30}H_{50}O_3$, $C_{30}H_{50}O_2$, and $C_{30}H_{50}O_3$, respectively. Analyses and the physical properties of a considerable number of derivatives of these products led Ochiai, Tsuda, and Kitagawa to conclude that the first three of their products were identical with those obtained by Miyasaka, and with three

of the four products obtained by Nozoye and Katsura. In a second communication Ochiai, Tsuda, and Kitagawa (325) reported the results of selenium dehydrogenation of the four sapogenins from which they concluded that the sapogenins obtained from soybean saponin by acid hydrolysis were triterpenoid alcohols, genetically related to each other, and to the many similar sapogenins belonging to the hederagenin series. Further confirmatory work on the structures of the sapogenols was reported by Tsuda and Kitagawa (437, 438) and by Tsuda and Ichikawa (436) which leaves no doubt concerning the triterpenoid hydropicenic nature of these compounds.

Iwasa (199) examined the saponin obtained as a by-product in the alcohol extraction method for the production of soybean oil. The product was a crystalline substance decomposing at 220-225° and gave, on hydrolysis with sulfuric acid, rhamnose, arabinose, glucuronic acid. and a sapogenin.

Phytosterolins: The second group of glycosides which are present in soybeans is represented by the phytosterolins or sterol glycosides. Jantzen and Gohdes (209) examined the white flocculent precipitate which separated at the interface when the saponification mixture from soybean oil was shaken with petroleum ether. From the crude product they isolated sitosteryl-d-glucoside. Glucose was identified in the form of its diphenylhydrazone after hydrolysis of the glucoside. The sterol was identified as sistosterol, but the melting point, 138.4 to 138.90, would seem to indicate that the product was in reality a mixture of sitosterols and stigmasterol. The same glucoside was isolated from crude soybean phosphatides which are especially rich in this material. According to Jantzen and Gohdes, crude soybean oil contains only about 0.03 percent of the sterol glucoside, whereas phosphatides prepared by washing the oil with water may contain up to 3.0 percent while the phosphatide-free oil will contain none at all.

Kondo and Mori (234) reported the isolation of sitosteryl-d-glucoside from soybean foots which were apparently produced as a by-product during the alcohol extraction of soybean oil by the Honen Oil Manufacturing Company. Soybean oil foots (12.0 kg.) were extracted with acetone and the insoluble portion successively extracted with methanol and ethyl ether. The insoluble residue was a tan-brown powder which consisted principally of phospholipids and phytosterolin. The phytosterolin was separated from the crude product by extraction with boiling 80 percent ethanol. On cooling the ethanol solution, sitosteryl-d-glucoside was obtained as a white powder, melting after recrystallization at 252-254° and corresponding in analysis to the formula, $C_{35}H_{60}O_6$. The tetraacetyl derivative was prepared by heating the product with pyridine and acetic anhydride. The recrystallized sitosteryl-d-glucoside tetraacetate was obtained as brilliant leaf-like crystals melting at 166-166.5° and corresponded to the formula $C_{43}H_{68}O_{10}$.

A portion of the sitosteryl-d-glucoside was hydrolyzed with amyl alcohol and hydrochloric acid and the free sterol recovered. After recrystallization from 80 percent ethanol, sitosterol was obtained in the form of cubic crystals melting at 138°. This product gave, on reduction with metallic sodium in amyl alcohol, dihydrositosterol melting at 175°. Glucose was recovered from the hydrolysate in the form of the phenylosazone melting at 205°.

Thornton, Kraybill, and Mitchell (426) prepared the mixed sterol glucosides from crude expeller oil by treating the oil with an adsorbent and subsequently extracting the glucosides from the adsorption agent. The sugar combined with the sterols was quantitatively recovered after hydrolysis of the glucosides and identified as d-glucose. The aglucone portion was found to consist of sterols similar to those which occur in the free state in soybean oil.

Isoflavone glycosides: The third group of carbohydrate-containing substances present in the soybean is represented by a series of yellow pigments of the isoflavone glycosides. Walz (445) isolated two yellow pigments from soybeans and proved them to be identical with the isoflavone glycosides, genistin and daidzin. By extracting 10 kg. of soybean meal with 90 percent methanol and working up the extract, he obtained 15 g. of genistin and 0.7 g. of daidzin. According to Walz, genistin, $C_{21}H_{20}O_{10}$, melted at 254-256° and had a rotation $(\alpha)_D^{21} = -27.70$ in 0.02 N NaOH. It gave a hexaacetyl derivative melting at 188°, a hexabenzoyl derivative melting at 132°, and trimethyl derivative melting with decomposition at 200-205°. Hydrolysis of the glycoside gave 1 mole of glucose and 1 mole of the aglycone, genistein, $C_{15}H_{10}O_5$.

Ochiai, Tsuda, and Kitagawa (324) likewise isolated the phenolic glycoside, genistin, but reported it to have a melting point of 298-300°. Previous to its isolation from soybeans, genistein was synthesized by Baker and Robinson (26, 27) who established it to be identical with prunetol and 5:7:4'-trihydroxyisoflavone.

Daidzin was found to melt at 234-236° and had a rotation $(\alpha)_D^{20} = -36.4°$. It crystallized with one molecule of water, gave an acetyl derivative melting at 203°, a benzoyl derivative melting at 145-150°, and monomethyl derivative melting at 206°. Hydrolysis yielded glucose and the aglycone, daidzein, which proved to be 7:4'-dihydroxyisoflavone, melting at 315-320°. The diacetyl derivative melted at 182° and the dimethyl derivative at 154°.

Okano and Beppu (330) isolated four different isoflavones from the alcoholic extract of soybeans. Lecithin was salted out from the solution and a crude saponin was precipitated from the alcoholic filtrate by the addition of acid. The saponin-pigment complex was dissolved in alcohol and the saponin precipitated by the addition of lead acetate. The lead-saponin complex was removed by filtration and the excess lead removed from the filtrate after which the pigments were fractionally separated. A mixture of ethanol and ethyl ether was added to the solution which resulted in the separation of yellow insoluble needles melting at 265°. The product corresponded to the formula $C_{21}H_{20}O_{10}$ and was named isogenistin. On hydrolysis glucose and an aglycone, isogenistein, melting at 302° were obtained. The aglycone corresponded to $C_{15}H_{10}O_5$ and gave a triacetyl derivative melting at 189° and a dimethyl-ether melting at 120-125°. On the basis of these results isogenistein was presumed to be 5,7,2'-trihydroxyisoflavone, isomeric with genistein.

The soluble portion yielded three other crystalline products. One of these which was designated as tatoin consisted of colorless needles melting at 318° having the formula $C_{16}H_{12}O_4$. It gave a diacetyl derivative melting at 185°,

a monomethyl-ether melting at 160-163° and a dimethyl-ether melting at 165°. These and many other reactions indicated the probable identity of tatoin with 8-methyl-5,4'-dihydroxyisoflavone.

The second product which was designated as methylgenistein consisted of faintly yellow needles melting at 298°. The product was characterized by the preparation and analysis of a large number of derivatives on the basis of which it was concluded methylgenistein was probably 8-methyl-5,7,4'-trihydroxyisoflavone.

The third product consisting of faintly yellow lustrous needles melting at 255° and corresponding to the formula $C_{22}H_{22}O_{10}$ was designated as methylisogenistin. On hydrolysis it yielded glucose and an aglucone melting at 301-302°, corresponding to the formula, $C_{16}H_{12}O_5$. The aglucone was converted into a series of derivatives, the analysis of which indicated it to be 8-methyl-5,7,2'-trihydroxyisoflavone, isomeric with methylgenistein.

PIGMENTS

The soybean presents a remarkable range of colors resulting from a diversity of pigments distributed throughout the various parts of the seed. The pigments responsible for the color of soybeans comprise four different types, namely the red-yellow carotenoids, the yellow isoflavone glycosides, the blue-purple anthocyanins, and chlorophyll.

Piper and Morse (349) have described this unusual variation in pigmentation as follows: "Most varieties of soybeans have unicolored seeds in the following colors: Straw yellow, olive yellow, green, olive, brown, and black, the last really a dark violet. Straw-yellow seeds are in some varieties very pale, especially when old, and are sometimes erroneously called white, but no truly white seeds are known in soybeans. In several varieties, like the Mammoth, the seeds have a greenish tinge if harvested before maturity, making it difficult to distinguish them from varieties whose fully mature seeds are greenish-yellow. The latter again merge by very fine gradations into olive and from this into brown. Bicolored seed occurs in but few varieties. The commonest are green or yellow with a saddle of black, the latter not sharply delimited. Some varieties have their seeds brindled brown and black, the two colors somewhat concentrically arranged. One variety has black seeds faintly marked with minute brown specks. On heterozygote plants the seeds are often irregularly bicolored and in some cases tricolored. Among recent introductions from Chosen (Korea) were several black and a few brown varieties with the outer layer of the testa broken by numerous cracks so as to expose the inner white layer. In the case of the black sorts this splitting has a net-like appearance, giving the beans a black and white color.

"The hilum or seed scar may be of the same color as the seed coat as in the case of black, brown, and a few yellow-seeded varieties or it may range from a pale brown to black in most straw-yellow, olive-yellow, and green-seeded sorts. In the latter case the color of the hilum is often of value in

distinguishing varieties having the same outer color. In a few varieties, as in Ito San, there is a minute brown spot on the micropyle which is diagnostic."

Despite this remarkable variation in color and its genetic significance, little effort appears to have been directed toward the isolation and identification of the individual pigments.

By means of microreactions with a series or reagents Kondo (233) examined the colored contents of the palisade cells and concluded that the blue color of the Japanese seed was an anthocyanin, the brown color of the black Chinese seed a tannin-containing anthocyanin, the olive-green color of the Japanese green seed chlorophyll, and the yellow-brown color of the dark wine-red Japanese seed probably tannin. He concluded that the palisade cells of yellow seeds contain no pigment, the color being located in the cotyledon. According to Alexandrov and Alexandrova (5) the basic type of pigmentation consists of a blue anthocyanin in the cell sap and of the chlorophyll of the plastids. According to these authors, the yellow coloration of the cell walls results from the presence of phlobaphene, but since the phlobaphenes are usually deep red in color it is more than likely that the yellow color has its origin in the previously mentioned isoflavone pigments. In addition to the pigments of the palisade cells, there are present in the oil droplets of the cotyledon various carotenoid pigments which impart to the expressed oil its characteristic reddish-yellow color. These pigments will be discussed under the sections on oil and vitamin A. Chlorophyll, which occurs in the plastids, will be discussed under oil properties since it affects the color of the oil removed from the seed during pressing or solvent extraction.

VITAMINS

The term vitamin is used to designate those substances, which in addition to fats, proteins, carbohydrates, and mineral salts, are required in very small amounts to maintain normal growth and health. Chemically there is no basis for placing vitamins in one group because, generally speaking, they are as unrelated as simple sugars, terpenes, pyridine, pyrimidines, and sterols and consequently no generalization can be made with respect to their chemical properties. They are, however, alike in one respect, namely, the absence from the diet of any one of the vitamins causes a specific disease.

Vitamin A: Vitamin A is a growth vitamin and prevents xerophthalmia or hardening of the membrane of the eye and cornea, hence is referred to as the anti-xerophthalmic vitamin. Certain carotenoids, particularly β-carotene, can be converted in the animal organism into true vitamin A which is stored in the liver often in considerable quantities.

Although a number of workers have reported the presence of vitamin A in soybeans or soybean oil, the question of the extent of its presence has not been satisfactorily settled. Most vitamin assays of vegetable oils in general indicate the presence of but little vitamin A or D. De Gironoli (98) who

was one of the earlier investigators of the vitamin A potency of soybeans concluded from a clinical study on infants that this oil contained no vitamin A. Scheunert (381) reported that soybean phosphatides contained no antirachitic vitamin and only negligible traces of vitamin A. Ohtomo (327) reported that soybean cake contained some vitamin A while Tso (435) stated that soybean milk was comparable to cows' milk in vitamin A and richer in vitamin B although inferior in mineral content. Wan (446) reinvestigated the dietary properties of soybean milk and concluded, contrary to the findings of Tso, that as measured by rat assays, soybean milk was inferior in vitamin A in comparison with cows' milk. A diet of soybean milk powder (1/3) and white wheat (2/3) with added salt did not result in as good growth even when supplemented with vitamins A, B, and D as did a similar diet containing cows' milk in the form of Klim. The poorer growth observed with soybean milk was attributed to protein deficiency in the case of the vitamin supplemented diet.

Hayward (170), however, states that examination of 50 varieties of soybeans showed the presence of 10 to 210 γ of carotene per 100 grams, equivalent to 0.16 to 3.5 international units of vitamin A per gram. Assays of expeller meal containing 8 percent fat and extracted soybean flour containing 3 percent fat indicated the presence of 0.36 and 0.30 international units of vitamin A, respectively, and 0.165 and 0.132 international units of vitamin D per gram. He concludes from these results that soybean flour or meal are not reliable sources of these vitamins for feeding livestock or poultry.

Lantzing and Van Veen (244) analyzed 16 samples of soybeans representing a number of different varieties for their content of provitamin A. The beans were ground, treated with alcohol, extracted with petroleum ether, and the extract allowed to separate into an epiphysical and a hypophysical phase. The composition of the two phases was determined by chromatographic adsorption of the pigments followed by spectrographic and colorimetric analysis.

It was found that the epiphysic phase consisted of β-carotene, whereas the hypophysic phase consisted of xanthophyll. The various samples of soybeans which were examined gave results varying between 40 and 80 γ of carotenoids per 100 grams or approximately 70 to 180 international units of provitamin A per 100 grams of ground beans. The β-carotene content was equivalent to 70 to 130 international units per 100 grams of ground beans.

Wilber, Hilton, and Hauge (462) reported that soybeans appeared to suppress the transference of vitamin A from dairy rations to the butter when soybeans constituted part of the feed. In a later communication these authors (169) concluded that there was present in soybean oil and soybean oil meal, obtained either by pressing or solvent extraction, an unknown factor which suppressed the transfer of vitamin A from the rations to butter and that prolonged extraction with ether and alcohol failed to completely remove this factor from the bean.

Frey, Schultz, and Light (127) found that shaking carotene solutions with ground soybeans in the presence of oxygen led to the complete decolorization of the carotene. When 0.09 ml. portions of carotene solution, equivalent to 272 U.S.P. units, after they had been shaken with a suspension of 10 mg. of active soybean in one ml. of water, were fed daily to assay animals, no

response was observed, whereas 0.001 ml. portions of untreated carotene solution equivalent to 3 U.S.P. units gave a normal growth response. These authors also reported that 99 percent of the vitamin A present in codliver oil was destroyed by treatment with active soybean. However, Kishlar (226) states that more than 100 assays have been made of carotene-soybean meal mixtures, and no abnormal destruction of carotene was found.

A recent investigation by the Bureau of Agricultural Chemistry and Engineering (230) has clarified the problem of the alleged destruction of vitamin A by the action of an oxidase in raw soybeans. A series of feeding experiments employing a raw-soybean diet and daily administration of the vitamin supplements apart from the diet, indicated that there was no marked destruction of the vitamins as a result of their contact with raw soybean meal, under the conditions of the experiments. It was concluded, therefore, that the unsatisfactory rate of growth with raw soybeans alone was attributable to a protein, rather than a vitamin, deficiency.

Sherman and Salmon (395) have reported the results of the determination of the carotene content of the large number of different varieties of green and mature soybeans. Most varieties of soybeans in the green stage were found to be very good sources of carotene with a range from 212 to 705 γ per 100-gram fresh sample. The carotene content of different varieties of mature soybeans ranged from 18 to 243 γ per 100-gram air-dry sample. Excessive weathering of soybeans, produced by allowing plants to stand in the field two or three weeks after the seed reached maturity, caused a destruction of as much as 50 percent of the carotene in several varieties of mature soybeans.

In a subsequent report Sherman (393) correlated the carotene content with biological assays for five varieties of soybeans. Chromatographic analyses of the biologically active carotenoid pigments extracted from the mature seeds demonstrated that the pigment contained in the purified extract was 80 to 88.8 percent β-carotene and 2.5 to 11.8 percent α-carotene. Cryptoxanthin was not found in any of the soybeans analyzed.

Sherman concluded that, "The carotene content of many varieties of soybeans at maturity is as great as that of yellow corn. In terms of total vitamin A activity, however, the yellow corn, because of the presence of relatively large amounts of cryptoxanthin, was superior to all varieties of mature soybeans studied." The superiority of yellow corn to soybeans as a source of vitamin A has been reported by Mitchell and Beadles (281).

Halverson and Sherwood (145) reported the vitamin A activity, obtained by means of rat-growth assays, in 15 samples representing 9 varieties of soybeans. They found the vitamin A content per gram of soybeans varied from 0 to 1.3 international units and concluded, therefore, that none of the varieties examined contained any appreciable quantity of vitamin A.

Snyder and Moore (401) determined the content of carotene in the whole soybean plant at weekly intervals from the time just prior to the formation of flowers until the stalks were completely mature and dry. The carotene content

was found to increase to a maximum of 432 micrograms per gram of dry matter by the time the pods began to fill and then decrease to 7.9 micrograms per gram of dry matter at final maturity.

Vitamin B complex: In view of the confusion existing in the literature relative to the nomenclature of the various components of the so-called vitamin B complex, it is necessary to discuss briefly its nomenclature prior to reviewing the published investigations on the occurrence of this nutritional factor in the soybean.

In the course of the early investigations on the cause and prevention of beriberi the terms water-soluble B, vitamin B, beriberi vitamin, and anti-neuritic vitamin were used by various authors to refer to the dietary factor essential to protect against the disease. As research on this factor continued it became evident that vitamin B was not a single substance but a complex group of chemical entities, the absence of one or more of which resulted in different pathological conditions in experimental animals.

A number of the factors of the B complex have now been isolated in pure form and their structures determined and verified by synthesis. The members of the B complex have been designated as follows 7/.

"Vitamin B_1 is a factor which prevents and cures beriberi in man and polyneuritis in animals. It has been isolated and synthesized and is now called thiamin. The crystalline synthetic compound is thiamin chloride. Vitamin B_1 is also aneurin.

"The term 'Vitamin B_2' has been used in various ways and now has no generally accepted meaning. It has been used to refer to the compound now known as riboflavin, to refer to all components of the vitamin B complex other than vitamin B_1, and in some instances, other individual components of the vitamin B complex.

"Nicotinic acid (nicotinamide or nicotinic acid amide) is synonymous with the pellagra-preventive factor, which is also specifically for the prevention or cure of blacktongue in dogs.

"Riboflavin, which has also been known as vitamin B_2 and G, is a compound necessary for growth in rats and chicks and which prevents a specific dermatitis and cataract in rats. It has also been shown to be effective in the treatment of lesions of the mouth of man, called cheilosis, as well as keratitis. It is a component of an oxidation-reduction system in living cells.

"Pantothenic acid - a compound necessary for growth in rats and chicks, and which prevents chick dermatosis.

7/ Nelson, E. M. Private communication to the authors dated May 17, 1940.

"Vitamin B_6 - also called pyridoxine, essential for preventing dermatosis in rats, and necessary for growth in rats and chicks.

"Vitamin B_3 - a factor necessary rapid gains in weight and normal nutrition of pigeons.

"Vitamin B_4 - a factor for prevention of a specific paralysis in rats and chicks.

"Vitamin B_5 - a factor necessary for maintenance of weight in pigeons.

"Factor W - a factor necessary for growth in rats."

Wan (446, 447) concluded from a comparative study of soybeans and milk that soybeans contain three times as much vitamin B_1, but only two-thirds as much vitamin B_2 (flavine), as does dried milk. Chen (79) concluded on the basis of rat assays that salted egg white, beef, and dried soybean curd were good sources of vitamin B_1. Miller and Robbins (278, 277) reported that cooked beans were a good source of vitamins A, B, and G but a poor source of vitamin C. Scheunert and Schieblich (382) found that fresh soybeans grown near Leipzig contained practically no vitamin A but did contain significant amounts of vitamin B_1 and vitamin B_2. The content of vitamin B_1 corresponded to about 1/40 of the amount found in a very good dried brewers' yeast, while that of B_2 corresponded to about 1/8. Consequently, 1 g. of fresh soybean contains the equivalent of about 1.5 international units of vitamin B_1. Hayward (170) reported that a sample of solvent-extracted meal assayed 2.8, and a hydraulic process meal 1.0 international units of vitamin B_1 per g. of solids. Mitchell and Beadles (282) found that 20 percent of soybeans in the diet furnished sufficient vitamin B for maximum growth and maintenance of health in rats. They concluded that 20 to 30 percent of soybeans in the diet was as effective as 70 percent or more of corn as a source of vitamin G, or that soybeans contained 2 to 3 times as much of this vitamin as does corn.

Sherwood and Halverson (392) reported soybean meal to be a good source of vitamin B. According to these authors, soybean meal contains 1.1 international units of vitamin B compared to 5.4 international units per gram for linseed meal. In a later communication Halverson and Sherwood (145) reported the examination of 15 samples representing 9 varieties of soybeans from which it was found that the vitamin B_1 content varied from 3.2 to 4.8 international units per gram.

Norris and coworkers (321, 463) have shown the essential nature of riboflavin in the diet of chicks and demonstrated its necessity for hatchability of fertile eggs. They introduced the term "Chick Unit" as a measure of riboflavin in the feed mixture which is roughly equivalent to 1 microgram of flavin. They determined that chicks need approximately 290 units of vitamin G per 100 grams of feed in order to attain normal weight at eight weeks age. Hens require about 230 units per 100 grams of feed, in order to produce eggs that will hatch well. As small an amount as 130 units per 100 grams of feed is sufficient for normal egg production. The quantity

of vitamin G found in soybean oil meal compared with other feedstuffs used in poultry feeding, as determined by these authors, are recorded in Table 9.

Table 9. Relative Vitamin G Content of Common Feedstuffs Used in Poultry Feeding

Feedstuffs	Units of Vitamin G per gram
Dried pork-liver	100
Dried yeast	35
Dried whey	30
Dried skimmilk	20
Alfalfa meal, dehydrated	16
Alfalfa meal	11
White fish meal	10
Sardine fish meal	7
Meat scrap	6
Menhaden fish meal	5
Wheat germ	4
Soybean oil meal	3
Wheat middlings, standard and flour	2
Wheat bran	2
Wheat red-dog flour	1
Yellow corn	1
Wheat, hard and soft	0.5
Barley	0.5
Buckwheat	0.5
Oatmeal	0.5
Oats	trace
Corn-gluten meal	0

Levine and Remington (253) report the presence of 2.4 to 3.2 Bourquin-Sherman vitamin G units per gram of soybeans. Kishlar (226) reported the presence of 2.5 Sherman rat units of vitamin G per gram of high grade expeller meal and concluded that soybean meal of good quality has approximately half the vitamin G content of dried skimmed milk and about twice the vitamin G content of 55 percent protein meat scrap or 60 percent fish meal, and 20 times as much as common cereals. These conclusions are at variance with the above-mentioned results reported by Norris and coworkers.

The flavin deficiency of expeller-pressed soybean meal in chick rations has also been demonstrated by Christiansen et al (84) . These authors conclude that "the protein of the grains supplemented solely by soybean oil meal is inefficient and requires additional supplementation with other protein for maximum efficiency." The protein materials which were effective in supplementing soybean oil meal were found to be those high in flavin which would indicate the observed deficiency resulted primarily from insufficient flavin rather than from a protein deficiency.

According to Schneider, Steenbock, and Platz (384) rat acrodynia can be cured either by vitamin B_6 or linoleic acid and vegetable oils containing this acid. Although soybean oil does not contain vitamin B_6 it does contain a very considerable amount of linoleic acid in the form of glycerides and may, therefore, be considered effective in curing acrodynia in rats. In this connection it should be mentioned that contrary to earlier reports no untoward symptoms were observed in growing chicks until the soybean oil content of the ration amounted to 10 percent or more (173).

Vitamin C: Vitamin C, or the antiscorbutic vitamin, is water-soluble and is found particularly in fresh fruits and certain green vegetables. It was the first vitamin to be obtained in crystalline form and its constitution has been proved by total synthesis. Chemically it is l-ascorbic acid, $C_6H_8O_6$. It is very sensitive to oxidation and heat, and is the most easily destroyed of all the known vitamins.

The literature relative to the occurrence of vitamin C in soybeans is not very extensive. Lee and Read (247) found that soybeans germinated in the dark at 25° formed ascorbic acid in increasing amounts up to about the tenth day. The maximum amount formed was nearly 0.4 mg. per seedling. The largest amounts were found in the cotyledons, and it was present mainly in the reversibly oxidized form. Sunlight caused a production of about twice the amount formed in the dark. Dry soybeans are reported to be relatively poor in vitamin C. Similar results were reported by Sugiura (409).

Vitamin D: Vitamin D, or the antirachitic vitamin, occurs in the liver. Before it was isolated and properly characterized, a different antirachitic principle was obtained by irradiating ergosterol with ultra-violet light. This second vitamin D has been obtained crystalline and is designated as calciferol or vitamin D_2. It is isomeric with ergosterol, one of the sterols contained in yeast.

Vitamin D from liver oil, vitamin D_3, is closely related to the product of irradiating ergosterol. Vitamin D_3 can also be derived from 7-dehydrocholesterol by exposing it to the ultra-violet light. Both calciferol (vitamin D_2) and vitamin D_3 are strongly antirachitic.

Heilbron, Kamm, and Morton (172) examined a wide variety of oils with respect to the possible presence of their pro-vitamin D absorption spectra. No evidence of absorption bands characteristic of these substances were found in soybean oil.

Bacharach (23) found that refined soybean oil was moderately rich in vitamin A but devoid of vitamin D. About the same time, Izume, Yoshimaru, and Kimatsubara (204) reported antirachitic properties for hydraulic-pressed, alcohol-extracted, and benzine-extracted soybean oils. They found that refining did not appreciably alter the activity of the oil, and that the activity was increased by irradiation. Irradiation of the unsaponifiable fraction produced well-defined absorption bands characteristic of ergosterol. In a later communication by Izume, et al (203), it was claimed that 20 percent of the unsaponifiable fraction of soybean oil consisted of ergosterol which was effective after irradiation in curing rachitis in mice in daily doses of 0.01 mg. compared to 0.1 mg. for the irradiated oil. Despite the reported vitamin D activity, extensive investigation of the sterol fraction has failed to reveal the presence of ergosterol in soybean oil.

Campos, Cavalcanti, and Santos (74) could find no evidence of the presence of an antirachitic factor in soybean oil.

Vitamin E: Vitamin E or the antisterility vitamin is fat soluble. Evans, Emerson, and Emerson (117) isolated, from wheat-germ and other vegetable oils, two alcohols, α- and β-tocopherol, having the formulas $C_{29}H_{50}O_2$ and $C_{28}H_{48}O_2$, respectively. These alcohols are oily liquids which form crystallizable derivatives. Both tocopherols possess vitamin E activity but the former is about 2.5 times as active as the latter. Deficiency of vitamin E in female animals renders them incapable of bearing viable offspring.

Sahashi (377) appears to have been the first to observe from the anatomical examination made on rats at the termination of feeding experiments that soybean oil possessed vitamin E activity. In a subsequent communication by Suzuki, Nakahara, and Sahashi (417) it was reported that when soybean oil was used as the only source of vitamin E, testicular degeneration was prevented in contrast to the marked degeneration which occurred when peanut, coconut, sesame, palm, and blubber oils were fed under the same conditions. The soybean oil used in these experiments was freshly expressed in the laboratory and had an iodine number of 142.5 which is considerably higher than most commercially produced oils in this country.

In a still later communication by the same authors (418) it was concluded on the basis of breeding tests with 7 albino female rats, that soybean oil contained antisterility vitamin E. However, the experimental evidence is not very convincing since 3 of the females never became pregnant; another gave birth to one litter of 4, none of which survived; still another became pregnant but died prior to completion of gestation; a sixth gave birth to one litter of 7 young, all of which died due to failure of lactation and a second pregnancy led to abortion; the seventh gave birth to 3 litters all of which died within 2 to 3 days due to failure of lactation. Based on the interruptions of gestation the amount of vitamin E in soybean oil was assumed to be relatively small.

In 1936 Goettsch and Pappenheimer (131) observed that certain vegetable oils including corn, cottonseed, peanut, and soybean, when added to a causative diet provided a protective factor against nutritional encephalomalacia in chicks. The disease was not thought to be a manifestation of vitamin E deficiency since addition of natural foodstuffs rich in vitamin E did not protect the chicks.

Coombes, Elvehjem, Phillips, and Hart (86), reported that encephalomalacia, a type of chick paralysis or crazy chick, was caused by an unknown nutritive factor in the ration, and that soybean oil but not cottonseed oil provided excellent protection against the disorder. Ni (320, 319) likewise reported that Chinese gelatin as well as soybean oil would prevent the disease and concluded therefore the factor was non-lipoidal in nature.

Jukes and Babcock (216) concluded on the basis of extensive feeding experiments that when alfalfa meal was added to the diet it was more active than soybean oil in improving growth and preventing paralysis in chicks. It was further concluded that there were two different forms of the same active factor since protection against paralysis was observed either by a fat-soluble fraction prepared from soybean oil or by a water-soluble fraction prepared from fat-extracted alfalfa meal.

Recently Pappenheimer, Goettsch, and Jungherr (346) have published a monographic report on their studies of nutritional encephalomalacia in chicks. The pathological changes resulting from feeding chicks a synthetic diet containing all the factors known to be necessary to normal rat nutrition except vitamin E were definitely described and defined, and the disease designated as nutritional encephalomalacia.

The protective factor was concentrated from soybean oil by extraction with 95 percent alcohol. It was found to be present in the unsaponifiable fraction when due precaution was taken against oxidation and the proper solvents were used for extraction. The fatty acid and water-soluble fractions were found to be inert. Protection was also afforded when α-tocopherol (vitamin E) from vegetable oils or synthetic dl-α-tocopherol acetate was included in the diet. The above results would seem to indicate that soybean oil does in fact contain vitamin E but at present the amount cannot be expressed in quantitative units.

Pappenheimer and Goettsch (345) observed that the addition of soybean oil to the diets of dystrophic rabbits resulted in the production of viable offspring which could be raised to maturity, whereas its omission from an otherwise similar diet of the parent resulted in death of the offspring within one day of birth. Autopsy showed the presence of lesions identical with those found in certain stages of adult nutritional muscular dystrophy.

Later it was found by Goettsch and Ritzmann (132) that naturally occurring α-tocopherol prevented the development of muscular dystrophy in young rats when it was fed from the tenth to the twenty-fifth day after birth, whereas control rats under similar conditions but without the supplement of α-tocopherol showed symptoms of muscular dystrophy at the end of the test period.

Mackenzie and McCollum (261) and also Morris (286) showed that natural α-tocopherol from wheat germ oil was effective in curing muscular dystrophy in rabbits and in addition Morris demonstrated that synthetic α-tocopherol was equally if not more efficacious than the natural product. He found the lower limit of a single curative dose to be about 20 milligrams of synthetic α-tocopherol per animal.

On the basis of the evidence mentioned above it may be assumed that (1) vitamin E is more than an antisterility factor; (2) soybean oil possesses this factor although to a much less marked degree than does wheat germ oil; and (3) the factor is contained in the unsaponifiable fraction of soybean oil from which it can be produced in concentrated form by extraction with suitable solvents.

Vitamin K: During the course of some experiments on the sterol metabolism of the chick, Dam (93, 94) observed that chicks raised on certain artificial diets became anemic, had large subcutaneous and intramuscular hemorrhages and in one chick the blood-clotting time was markedly increased. Later he reported (77) that the hemorrhagic condition was not relieved by the addition to the diet of vitamin C or any other of the then known vitamins of essential dietary factors. He suggested that the hemorrhages were due to the lack of a new fat-soluble factor, which he named vitamin K.

It has been shown that this antihemorrhagic factor is widely distributed in green leaves and vegetables and is especially abundant in dried chestnuts, spinach, cabbage, and alfalfa leaves. Almquist and Stokstad (8, 7) reported in 1935 that the addition of 0.5 percent of dry alfalfa to the deficient diet prevented the appearance of symptoms, and since that time alfalfa has been one of the main sources of this vitamin.

Almquist and Stokstad (10) were the first to report the presence of vitamin K in soybean oil. The blood clotting time of 2 weeks old chicks fed a diet supplemented with a hexane extract of alfalfa was 1.7 minutes; with one percent soybean oil 15.6 minutes; with 2 percent soybean oil 3.7 minutes; with 5 percent soybean oil 1.3 minutes, compared with the control group having a blood clotting time of 12 minutes. It was concluded (407) therefore that 2 to 3 percent of soybean oil added to the chick diet provided an adequate level of vitamin K.

Dam and Glavind (95) determined the amount of vitamin K in many types of plants. Soybean seeds dried in vacuum dessicators at room temperature were found to contain 25 units of vitamin K per gram of dry material. The method of assay was that described by Schnheyder (386) and developed by Dam and Lewis (79).

Evidence for the existence of a fat-soluble factor required by the chick for the integrity of the gizzard lining has been presented by Almquist and Stokstad (9). Almquist (6) showed that the gizzard factor was present in soybean oil and to a less extent in soybean meal. He also demonstrated the instability of the factor to heat and othyl alcohol by replacing an active soybean oil with the same oil after heating with ethyl alcohol or by heating the oil in vacuo for 24 hours at 120° whereupon it was observed that the activity of the protective factor was markedly reduced or completely destroyed. The gizzard factor does not appear to be identical with vitamin K.

OIL AND OIL-SOLUBLE CONSTITUENTS

Soybeans normally contain from 13.5 to 24 percent of lipids extractable by fat solvents such as ethyl ether, hydrocarbons, chlorinated hydrocarbons, alcohols, or carbon disulfide. Over ninety-five percent of this fatty material is found in the cotyledons and the remainder in the germ and seed coat. The oil or fat obtained by extraction of the seed consists principally of the glycerides of saturated and unsaturated fatty acids, admixed with a number of other lipid materials. The latter include the phosphatides, sterols, long chain hydrocarbons, alcohols and ketones, free fatty acids, pigments, vitamins, and antioxidants, as well as small amounts of nonlipid gummy or mucilaginous substances. The type and amount of the various lipids which may be present in the oil depend somewhat on the variety of seed, environment during growth, methods of harvesting, handling and storage of the beans, and especially on the manner of processing the seed and subsequent refining of the oil. Probably no commercially important vegetable oil is produced by the variety of processing practices to which soybeans are subjected.

It is not surprising, therefore, that the physical and chemical characteristics of crude soybean oils show considerable variations resulting from combinations of the various factors enumerated above. Individual oils derived from specific varieties of seed may show wide variations in physical properties and chemical compositions, but commercial soybean oils are much more uniform owing to the mixing of different lots of seed, or of different batches of oil during processing. Furthermore, the numerous refining operations tend to eliminate much of the secondary materials, especially gums, phosphatides, free fatty acids, unsaponifiable matter, and pigments.

The existing literature pertaining to soybean oil is voluminous and widely scattered; also much of it is superficial with respect to objective and execution. Systematic and comprehensive examinations of individual soybean oils with respect to all or even a considerable number of their physical and chemical properties are not extensive. Data relative to the variations of such properties as specific gravity, viscosity, refractive index, and specific heat, as a function of temperature, pressure, or other variables are either non-extensive or non-existant. Systematic and comprehensive chemical analyses, especially with respect to the non-glyceride constituents of the oil, are similarly non-extensive. Apparently, most of the investigations reported in the literature refer to laboratory preparations, usually solvent-extracted crude oils, and in many cases the variety or previous history of the seed is unknown or is not recorded. No doubt, many industrial concerns have amassed considerable data relative to oils produced in their own plants, but for the most part such information is not available in the scientific and technical literature.

Physical and Chemical Characteristics of Soybeans Oils

Most vegetable oils, and soybean oil is no exception to the general rule, are evaluated as to quality, freedom from associated impurities or added adulterants, adaptability to various processes or uses, and for other purposes, by means of certain inherent physical and chemical characteristics or properties. Some of these properties have been made the basis of trading rules for buying and selling oils and procedures for their determination are set forth in so-called official methods. Large numbers of determinations of these more common properties are made by and for oil mills, refiners and consuming industries.

Other properties, especially those of importance in highly specialized applications or processes, are determined with less frequency and often by arbitrary or improvised methods or under conditions applicable to or closely simulating the process in which the oil is to be employed. In some cases the results of these determinations are published but more often they are not.

In assembling the available data on the physical and chemical characteristics of soybean oil as has been done in the following pages several defects are evident, notably the narrow range of environmental conditions over which a given property has been measured; the lack of specificity of the material examined; the variety of methods which have been used by different investigators; and the variety of units in which the results have been expressed.

In the following enumeration of the chemical and physical properties of soybean oil an effort has been made to include, wherever available, information concerning the type or origin of the oil and the method applied in determining a given property.

Acetyl value: See hydroxyl number.

Acid value: See free fatty acids.

Break: The so-called "break" material found in most crude soybean oils consists principally of phosphatides, pigments, and mucilaginous materials which are removed from the seeds along with the oil. The various methods of pressing and extracting soybeans result in marked differences in the amount of extraneous or nonglyceride material which accompanies the oil. The temperature and pressure of pressing and the temperature, moisture content, and type of solvent influence the quantity and composition of nonglyceride substances which are removed from the seed. Similarly, the method and degree of clarification (settling, filtering, centrifuging, or washing) likewise affect the quantity of extraneous materials which remain in the crude oil. Where the phosphatides are to be recovered, their presence in the crude oil is desirable and advantageous, but much of the oil, especially in America, enters directly into the edible or drying oil industry where excessive "break" material is objectionable because it leads to increased refining losses.

The percentage of "break" material is determined by the modified Gardner acid-heat-break method (11) which consists essentially of adding 3 drops of 12 m. hydrochloric acid to a 25 gram sample of oil, heating rapidly to 288°, cooling, and diluting the sample with carbon tetrachloride. The "break" material which separates is filtered, washed, dried, and weighed. The amount of "break" material obtained under these conditions normally varies from 0.04 to 0.20 percent for extracted oils and from 0.20 to 1.00 percent for expeller oils. It was originally supposed that the amount of "break" material found by the heat-break method was correlated with the loss sustained in refining the oil. Such a relation exists with respect to many soybean oils, but numerous exceptions are found (Table 10) especially in the case of solvent-extracted oils. (See Table 10 on page 37).

The standard specifications (312) governing the purchase and sale of domestic, crude soybean oil impose a maximum limit of 0.60 percent on the content of "break" material and provide a penalty of 0.1 percent of the purchase price for each 0.1 percent in excess thereof.

Color: The color of soybean oil is usually determined by matching in a tintometer a column of oil, 25 mm. deep in the case of crude oils and 133 mm. deep in the case of refined oils, against a combination of red and yellow Lovibond color glasses. The results are expressed in terms of red and yellow units on the Priest-Gibson (N") color scale (129, 130, 259).

Although satisfactory for many purposes color values obtained by matching an oil against standard yellow and red glasses do not reveal the complete color characteristics of an oil. In order to obtain a true measure of the color of an oil it is necessary to know its absorption or transmittance characteristics over the entire visible spectral range. Transmittance curves (figures 2, 3 and 4) of a number of typical crude and refined soybean oils are produced from the data of Bickford, Anderson, and Markley (34). Inspection of these curves and reference to others in the original publication from which they are reproduced provides ample evidence of the manner in which soybean oils may vary in their color characteristics.

The color of crude soybean oil may vary considerably with the source of seed and the method of producing the oil but normally a 133 mm. column of oil is found to range from 70 yellow and 10 red units to 70 yellow and 30 red units on the N" color scale. Occasionally the color of a crude oil may depart from these values if, for example, the soybeans from which it is produced are immature and contain unduly large amounts of chlorophyll, or when the oil is overheated or otherwise improperly treated during processing. Also oil produced from green, brown, and black varieties of soybeans will produce abnormally colored oils if the colored seed coat is not removed before processing for oil.

Refined oils may vary appreciably in color depending on the use for which they are intended. For example, in mayonnaise or salad dressings a bright yellow oil may be desired, whereas for use in shortening, light colored varnishes, or synthetic resins a minimum of color is desired. Highly refined, edible quality soybean oil is generally produced with a color of 15 to 35

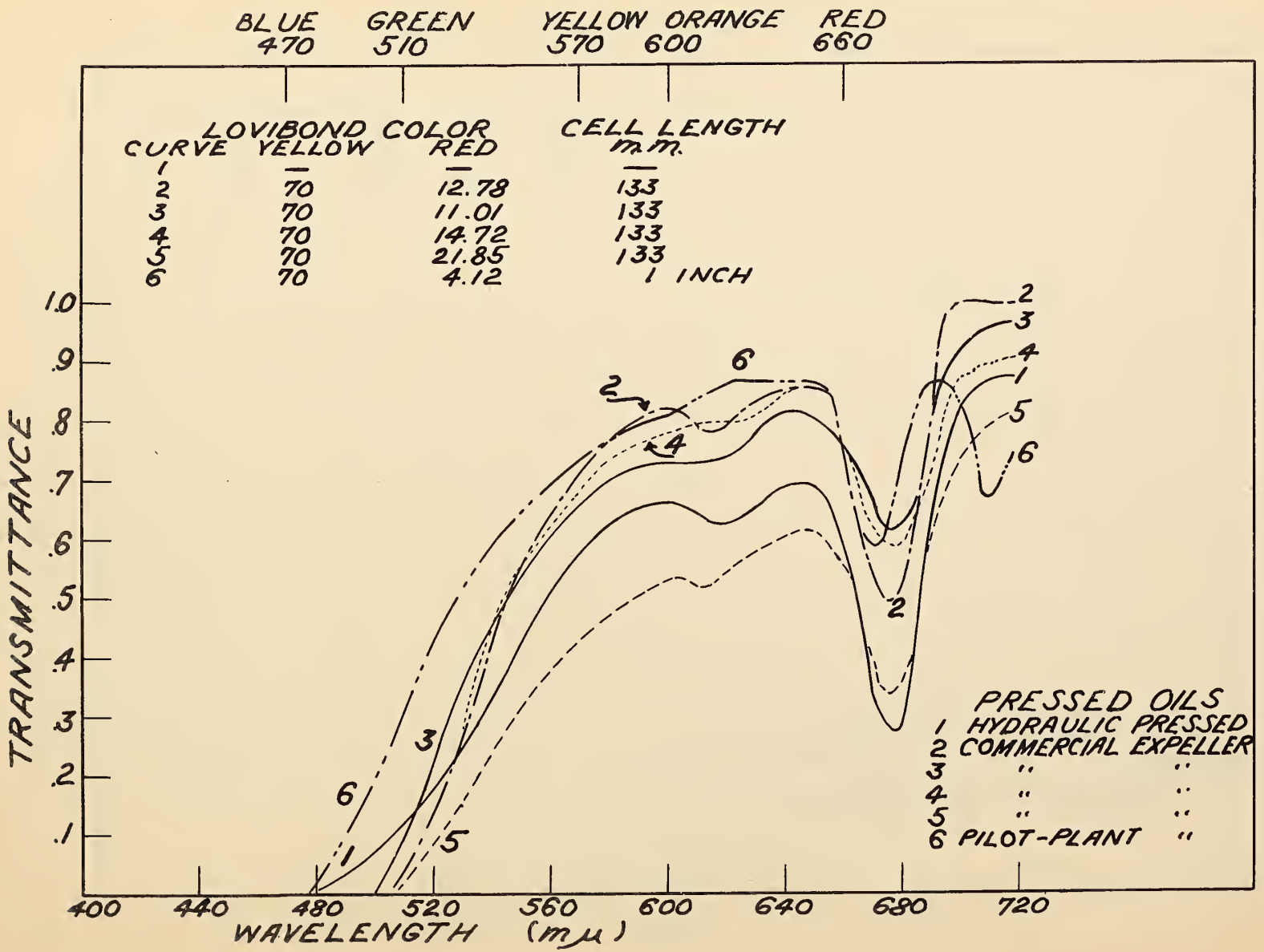


Figure 2.- Spectral transmittance of crude pressed soybean oils.

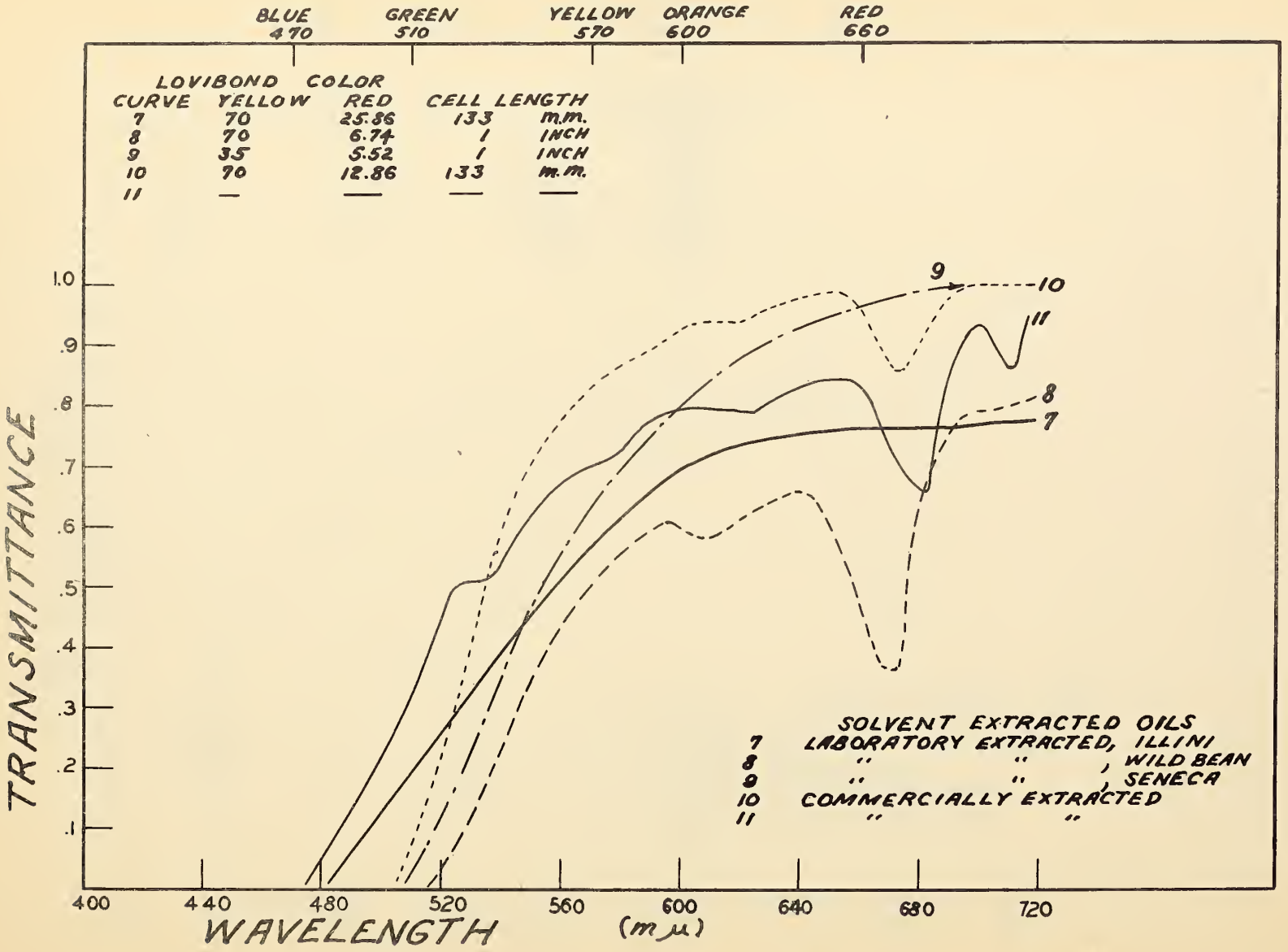


Figure 3.- Spectral transmittance of crude solvent-extracted soybean oils.

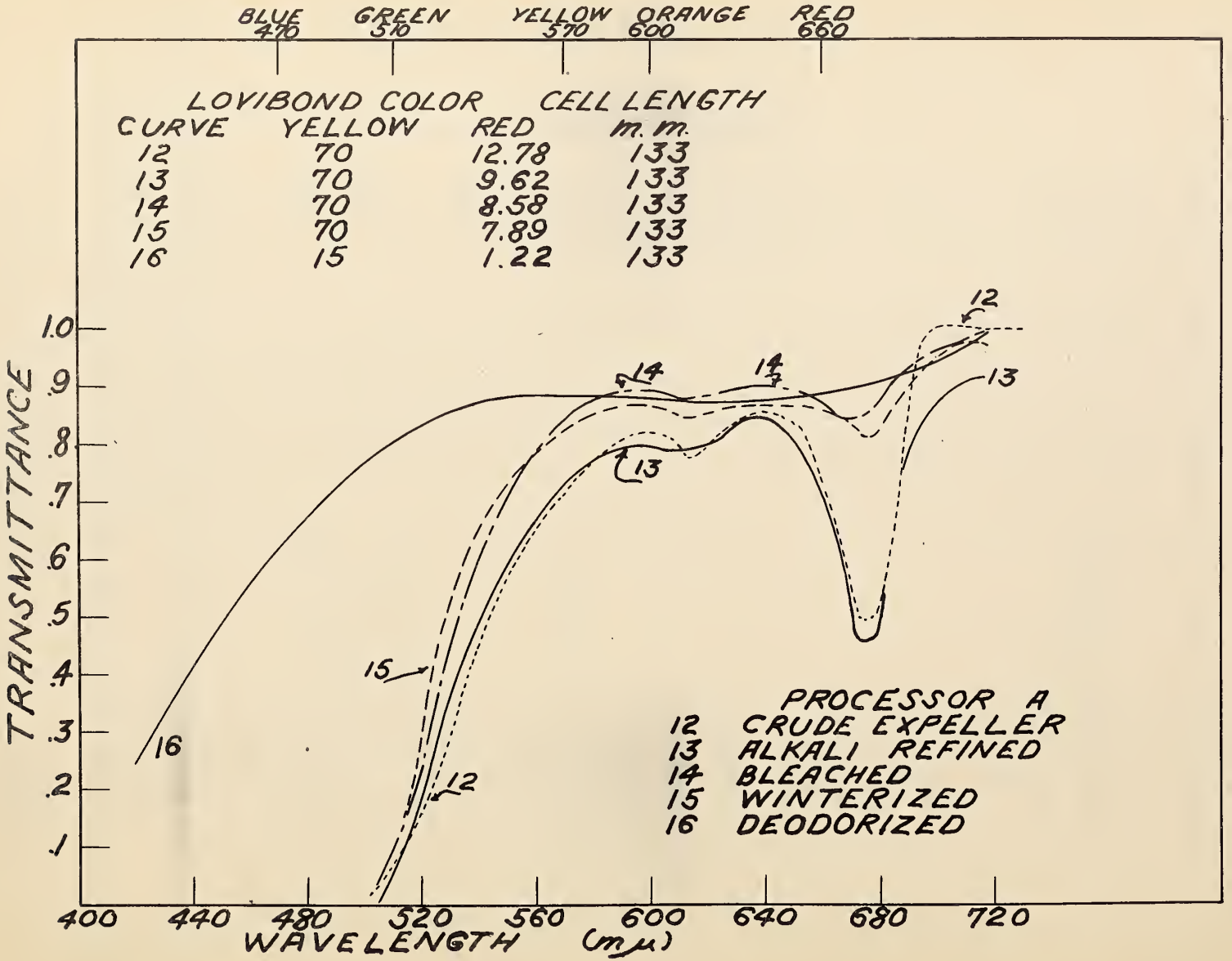


Figure 4.- Spectral transmittance of a soybean oil at various stages of processing.

Table 10. Factors Affecting the Refining Loss of Crude Soybean Oils

Sample No.	Type of oil	Free fatty acid	Refining loss	Break	Phosphorus
		Percent	Percent	Percent	Percent
48	Expeller-pressed	0.35	3.7	0.26	0.097
49	Expeller-pressed, roasted beans	0.52	6.5	0.58	---
56	Expeller-pressed, commercial	0.61	3.9	0.06	0.059
57	Commercial-pressed, clarified	0.45	4.3	0.15	0.021
59	Expeller-pressed	0.87	4.2	0.30	0.046
60	Expeller-pressed	0.42	3.3	0.26	0.043
61	Expeller-pressed, commercial	0.60	5.1	0.48	0.083
62	Expeller-pressed	1.02	5.4	0.36	0.058
63	Expeller-pressed	0.91	4.8	0.26	0.054
64	Expeller-pressed	0.75	4.1	0.28	0.044
65	Expeller-pressed	0.73	4.2	0.28	0.044
66	Expeller-pressed	0.81	4.1	0.24	0.042
67	Expeller-pressed	1.10	4.9	0.32	0.050
68	Expeller-pressed	0.78	3.9	0.26	0.044
69	Expeller-pressed	0.86	4.2	0.24	0.038
70	Cold pressed, commercial	0.43	4.3	0.37	---
72	Solvent-extracted, commercial	1.65	6.8	0.07	0.012
73	Expeller-pressed	0.83	4.8	0.26	0.045
74	Expeller-pressed	0.84	4.3	0.23	0.041
75	Expeller-pressed	0.86	4.0	0.25	0.041
80	Solvent-extracted, commercial	0.35	3.0	0.34	0.084

yellow units and 1.0 to 3.0 red units on the N" Lovibond scale when measured in a 133 mm. cell. When hydrogenated for use in shortening the color is further reduced to approximately 10.0 yellow units and 1.0 or less red units. Commercial varnish grade soybean oil is often sold on a guaranteed color of 30 yellow and 3 to 5 red on the Lovibond scale.

In Table 11 there are recorded data obtained at the Regional Soybean Industrial Products Laboratory which illustrate the normal variation observed in the color of various grades and types of soybean oils.

Table 11. Color Variation of Soybean Oils in Priest-Gibson (N") Color Units

Type of oil	Number of samples	Low	High	Cell length mm.
Expeller-pressed crude	23	70Y - 4.04R	70Y -10.03R	25
Expeller-pressed crude	7	70Y -13.16R	70Y -30.75R	133
Solvent-extracted crude	7	70Y - 3.5 R	70Y - 6.64R	25
Solvent-extracted crude	7	70Y -12.60R	70Y -29.35R	133
Alkali-refined	24	70Y - 6.34R	70Y -11.42R	133
Completely processed	13	10Y - 0.92R	35Y - 3.17R	133

Congealing temperature: The temperature at which soybean oil freezes or congeals has been reported to vary from -8.0 to -18° C.

Density: The density of soybean oil has been variously reported to range from 0.922 to 0.934 at 15° C. and 0.915 to 0.920 at 25° C. The specific gravity at 25°/25° for seven samples of solvent extracted crude soybean oil which were examined at the United States Regional Soybean Industrial Products Laboratory were found to vary from 0.9159 to 0.9219. Johnstone, Spoor, and Goss (211) reported the density in grams per cubic centimeter of a completely refined soybean oil at 25°, 37.8°, and 50° C. to be 0.9171, 0.9087 and 0.9004, respectively. These authors have also reported data for the density of hexane-, ethylene dichloride-, and trichloroethylene-soybean oil solutions throughout their miscibility range and for temperatures of 25°, 37.8°, and 50° C.

The density of soybean oil has been related to other physical properties as for example iodine number. These relationships are discussed under the correlated property.

Diene numbers: The diene number was originally proposed as a measure of the extent to which the double bonds of the fatty acid glycerides of vegetable oils existed in the conjugated state. Diene numbers ranging from 8.5 to 8.9 have been reported by Kaufmann, Baltes, and Büter (223) for soybean oils. Bickford, Dollear, and Markley (36, 37) were unable to confirm these results except in the case of blown soybean oils. The latter authors found that good quality refined oils had diene values ranging from 0.7 to 2.0. Furthermore, they established the fact that the presence in fats and oils of hydroxyl groups, peroxides, and possibly other oxidation products influences the magnitude of the diene number, apparently as a result of the reaction of these substances with maleic anhydride under the conditions specified for the determination of diene numbers.

Fatty acids: (See also titer value). The mixed fatty acids obtained by saponifying soybean oil have been reported to melt from 20° to 29° and solidify at 14° to 25°. The mean molecular weight of the mixed fatty acids is about 290, and the iodine number has been found to vary from 106 to 158.

Flash and fire points: Detwiler and Markley (101) examined a number of representative soybean oils with respect to their smoke, flash, and fire points. Average values which were found by the Cleveland open cup method for a number of typical soybean oils are given in Table 12.

Table 12. Average Temperatures of the Smoke, Flash, and Fire Points of Soybean Oils.

Type of soybean oil	Average smoke point		Average flash point		Average fire point	
	°F.	°C.	°F.	°C.	°F.	°C.
Expeller-pressed crude	380	183	573	300	664	351
Solvent-extracted crude	411	210	602	316	672	355
Alkali-refined	467	242	624	329	681	361

The results obtained by these authors indicated that expeller-pressed crude soybean oils generally exhibit lower smoke, flash, and fire points than do mechanically refined or alkali-refined soybean oils. Solvent-extracted crude oil likewise appeared to be superior to expeller-pressed crude oil with respect to these properties. Alkali refining was found to result in improvement of the thermal stability of soybean oil, whereas subsequent processing such as bleaching and deodorization, had little additional effect on this property. It was concluded that good quality soybean oil, free from excessive quantities of free fatty acids and foots, usually could be heated with safety to 600° F. (315° C.) and in some cases even slightly above this temperature without undue risk of fire.

Fluorescence: Most vegetable oils when subjected to ultraviolet irradiation emit fluorescent light which imparts to them color qualities different from those observed in ordinary light. For example, irradiation of soybean oil by a quartz light produces a light lilac fluorescence (142). Marcille (263) found that soybean oil like olive, peanut, and other seed oils exhibits a violet fluorescence and concluded that although some oils as found will fluoresce when irradiated with ultraviolet light, for the most part fluorescence is an acquired characteristic and is dependent on the treatment to which it is subjected during the various processing operations.

Free fatty acids: Soybean oils contain variable amounts of free fatty acids. In the case of freshly produced crude oils these acids may have pre-existed in the uncombined form in the seed at the time they were harvested, or they may have developed in the seed after harvesting, and before processing. Their presence in aged oils may be attributed to the above causes and also to hydrolysis of the glycerides after their extraction from the seed. Regardless of the manner in which the free fatty acids originated it is of importance to know the amount of these substances which is present in the oil.

The term used to express the quantity of free fatty acids which are present in an oil is known as the acid number. It is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of an oil. Examination of a large number of freshly produced soybean oils have shown variations in acid number as follows: Solvent-extracted oils 0.35 to 3.85; expeller-pressed oils 0.86 to 2.10.

Alkali refining results in a reduction of the free fatty acid content and carefully refined and deodorized soybean oil should have an acid number of 0.10 or less. However, freshly refined and deodorized soybean oils have been produced with acid numbers as great as 0.40. The acid number changes with conditions which tend to produce hydrolysis or splitting of the glycerides and in due course of time and under adverse storage conditions the acid number may become relatively large.

Instead of acid number the trade prefers to express the content of free fatty acids in an oil on a percentage basis, which in the case of soybean oil is calculated as oleic acid. Trading rules (312) limit the maximum free fatty acid content of crude soybean oil to 1.5 percent, and impose a penalty of 0.5 percent of the purchase price for each 0.5 percent in excess thereof.

Hehner number: The Hehner number expresses the percentage of water insoluble acids which are found in an oil after saponification, neutralization, and extraction of the liberated acids with water. Values of 93.0 to 94.3 have been reported for soybean oils (119).

Hexabromide number: When the fatty acids prepared from soybean oil are brominated in ether solution an insoluble precipitate of hexabromostearic acid is obtained which when freed from impurities melts at 180-181°. The weight of the precipitate times 100 divided by the weight of fatty acids gives the percentage of hexabromides or the hexabromide number. Hexabromide numbers of soybean oils varying from 2.2 to 9.65 have been reported by Steele and Washburn (403), Bailey, and Baldsiefen (24), Kaufmann (222) and others.

These values were once presumed to represent the relative percentages of linolenic acid present in soybean oils but it has been shown by Shinowara and Brown (396) and Kaufmann (222) that the yield of hexabromides varies with the composition of the other fatty acids present in the mixture and is therefore not a reliable index of the content of linolenic acid.

Hydroxyl number: The hydroxyl number and the related acetyl value are intended to measure the extent to which hydroxyl groups are present in an oil, presumably in the form of the glycerides of hydroxy acids. In a freshly refined oil such groups may represent naturally occurring hydroxy acid glycerides but in aged oils hydroxyl groups may be present as a result of the oxidation of unsaturated acids or hydrolysis of triglycerides to mono- and diglycerides.

The hydroxyl number is defined as the number of milligrams of potassium hydroxide equivalent to the hydroxyl content of the sample of oil, whereas the acetyl value is defined as the number of milligrams of potassium hydroxide required to neutralize the acetic acid produced on saponification of one gram of acetylated oil. Both values are found to vary with the method used in their respective determinations. Acetyl values for soybean oil varying from 4.9 to 20 have been reported in the literature but the higher values are improbable except in the case of aged or oxidized oils.

The hydroxyl number of a series of crude, semi-refined, and completely refined soybean oils obtained from soybeans of different varieties and grown under different soil and climatic conditions were determined at the Regional Soybean Industrial Products Laboratory by the method of West, Hoagland, and Curtis (451). The values found for these oils varied from 2.6 to 6.7. Mild oxidation of a freshly refined, edible grade soybean oil by aeration at 98° C. in a Swift stability apparatus was found to result in a gradual increase in the hydroxyl number as follows: Time of aeration 0, 6, 8, and 10 hours; hydroxyl number 3.9, 4.2, 6.1, and 11.5, respectively. The increase in hydroxyl number results from the oxidation of the double bonds of the unsaturated acids present in the form of glycerides.

Iodine number: The iodine number is defined as the number of grams of iodine which 100 grams of fat or oil is capable of absorbing. Although varying somewhat with the method and conditions under which it is determined it provides information concerning the total unsaturation of most vegetable oils containing non-conjugated ethenoid linkages. The so-called official Wijs method is generally accepted as most reliable for use with soybean oils. Examination at the Regional Soybean Industrial Products Laboratory of hundreds of samples of commercial and laboratory processed oils, including crude, semi-refined, and completely refined oils derived from many varieties of soybeans grown under different soil and climatic conditions has revealed iodine numbers ranging from 103 to 151. The majority of commercial soybean oils were found to have iodine values within the range 130±3. Crude oils and the corresponding refined soybean oils differ only slightly in their respective iodine numbers.

Soybean oil of a minimum iodine value is preferred by the edible oil trade as it is believed that increased unsaturation is detrimental to keeping quality and also because the greater the degree of unsaturation the greater is the consumption of hydrogen during the hydrogenation or hardening process. On the other hand the drying oil trade prefers soybean oil of maximum iodine value because superior drying and film-forming properties are associated with a high degree of unsaturation.

Various relationships have been established between the iodine number and other physical properties of soybean oil, as for example iodine number and density, and iodine number and refractive index. Yokota and Tachimori (476) have shown that the relation between the density and the iodine number of soybean oils, is nearly constant. For the relation between the iodine number and refractive index of soybean oil see section on refractive index.

Optical rotation: Most fats and oils show very slight optical rotation unless they contain acids with monovalent polargroups such as are present in castor oil. Such optical activity as they may possess is generally attributed to the sterols which are normally present in the oil in small amounts. However, the optical behavior observed by Suzuki, Inoue, and Hata (413) is difficult to explain on this assumption. These authors observed that a number of freshly produced vegetable oils including soybean oil possessed a small optical rotation which gradually decreased to zero on standing. Soybean oil for example when freshly isolated was found to possess an optical rotation of +0.25 which gradually and regularly decreased to zero in the course of a few days. Since crude soybean oil contains up to 0.3 percent or more of mixed sterols it is to be expected that the oil might have a slight optical rotation but since these sterols are quite stable and do not change appreciably with age their presence offers no explanation of the observed decrease in rotatory power on standing.

Refining loss: Refining losses are caused by the presence of free fatty acids, phosphatides, and extraneous nonglyceride materials which are present in the crude oil. The apparatus and conditions for the laboratory determination of the refining loss for expeller, hydraulic, and extracted soybean oils are set forth in the Official and Tentative Methods of the American Oil Chemists' Society (11). Examples of typical laboratory refining losses are recorded in the annual reports of the Refining Loss Committee of the American Oil Chemists' Society (12).

Refractive index: When a beam of light passes from one transparent medium to another it is deviated or refracted and the ratio of the sines of the angles of the incident and of refracted light is a constant at the boundary between the two media. This constant ratio for any two given media, one of which is usually a glass prism, is known as the refractive index. It is an important property of fats and oils not because of any direct interest in the refraction of light by the lipid but because the index of refraction is relatively easy to determine, and is characteristic of a given oil, and because it can be correlated with other less easily determined properties.

The refractive indices of hundreds of samples of oil derived from soybeans representing many different varietal and cultural conditions have been determined over a period of several years at the Regional Soybean Industrial

Products Laboratory. These refractive indices have been found to range from $N_D^{25} = 1.47084$ to $N_D^{25} = 1.47423$.

Considerable effort has been devoted to correlating the refractive index with other properties of soybean oil, such as iodine number, density, and the oil content of the seed.

Pickering and Cowlishaw (348) were probably the first to establish a quantitative relationship between the refractive index and iodine number of soybean oil. They found as the result of the examination of hundreds of samples of fresh soybean, linseed, refined cottonseed, and peanut oils that the refractive index could be related to its respective iodine number by the equation: $N_D^{40} = 1.4515 + 0.0001171$ I.V. It was stated that the equation applied only to fresh oils, free from oxidation, and having a low content of free fatty acid and unsaponifiable matter.

More recently Majors and Milner (262) correlated the results of the determinations of refractive indices and iodine numbers of a very large number of soybean oils and studied the effects of variation of different factors on the accuracy of the correlation. These factors included among others the method of obtaining the oil from the seed, age of the sample of seed, the content of free fatty acids and of unsaponifiable matter of the oil.

These authors established the fact that the correlation curves varied from year to year but were remarkably constant for a given crop year. They concluded that the iodine number of soybean oil can be determined by calculation from the refractive index with an accuracy comparable to any chemical method, provided that the refractometer used is sufficiently accurate, the measurements are carefully made, and that for any given crop year, a correlations curve is established.

Leithe and Müller (248) devised a method for determining the percentage of oil in soybeans based on the differences of the refractive indices of the solution of the oil and that of the pure solvent.

Zeleny and Neustadt (477) investigated the determination of the oil content of soybeans by refractometric and solvent extraction methods. They proposed a method for the determination of the oil content of soybean seed based on the refractive index of a mixture of soybean oil having a refraction index, $N_D^{25} = 1.47302$, and a solvent consisting of halowax (α-chloronaphthalene) and α-bromonaphthalene adjusted to have a refractive index, $N_D^{25} = 1.63940$. For oils deviating in refractive indices from the standard oil they constructed a table of corrections to be added to or subtracted from the observed values.

Johnstone, Spoor, and Goss (211) published data for the refractive indices of various soybean oil-solvent solutions over their entire miscibility range at 25° C. The solvents comprised hexane, ethylene dichloride, and trichoroethylene and the oil represented a highly refined edible grade.

Reichert-Meissl number: The Reichert-Meissl number is defined as the number of ml. of 0.1 N potassium hydroxide solution required to neutralize the water soluble volatile fatty acids obtained from 5 grams of fat or oil. Values

ranging from 0.2 to 2.8 have been reported for soybean oil. It is not an important characteristic of soybean oil and is only seldom determined.

Saponification number: The saponification number or Koettstorfer number is defined as the number of milligrams of potassium hydroxide required to saponify one gram of fat or oil. The saponification number of freshly produced, solvent-extracted crude soybean oils which were examined at the Regional Soybean Industrial Products Laboratory were found to vary from 188.8 to 193.5.

Smoke point: See flash and fire point.

Specific heat: The specific heat of a sample of soybean oil having an iodine number of 134 and an acid number of 2.3 was determined by Long, Reynolds, and Napravnik (254) over a temperature range of 75 to 287° C. (167 to 548.6° F.). The data are given in Table 13. (See Table 13 on page 45).

Thiocyanogen number: The thiocyanogen number determination was proposed by H. P. Kaufmann (220, 221) on the supposition that the thiocyanate radical added quantitatively to but one of the two double bonds of linoleic acid and to but two of the three double bonds of linolenic acid, whereas iodine and other halogens added quantitatively to both of the double bonds of linoleic acid and all three of the double bonds of linolenic acid. On this assumption it, therefore, became possible to calculate the composition of the unsaturated acid fraction of fats and oils from the determined values for the iodine and thiocyanogen numbers.

Unfortunately for the accuracy of the fatty acid composition calculated in this manner, it has since been shown by Riemenschneider and Wheeler (372), Kass, Loeb, Norris, and Burr (218) and Hilditch and Murti (181) that the addition of the thiocyanate radical is far from quantitative for linoleic and linolenic acids and esters under the conditions of the Kaufmann method. However, by substituting in the equations used for calculation new constants obtained experimentally on highly purified linoleic and linolenic acids, it is possible to obtain accurate values for the composition of many fats and oils including soybean oils. The last mentioned authors presented data for the different values obtained in the unsaturated acid fraction of soybean oil when calculated by the use of the original Kaufmann constants and the revised constants. Higher values were found for linoleic acid and lower values for oleic and linolenic acids when calculated by the new constants as compared with those calculated by Kaufmann's constants.

A very considerable number of soybean oils representing many varieties of seed and types of processing have been examined at the Regional Soybean Industrial Products Laboratory. Variations of from 78.0 to 90.5 were observed in thiocyanogen numbers compared to 103 to 151 for the iodine numbers of the same oils.

Titer: The solidification point of the mixture of fatty acids and unsaponifiable matter obtained by saponifying, acidulating, and washing the water-insoluble fraction of fats and oils is known as the titer. It is determined

Table 13. Specific Heat of Soybean Oil

Temperature		Specific Heat
°C.	°F.	Cal./Gram
75	167	0.568
85	185	0.579
130	266	0.582
140	284	0.591
140	284	0.606
151	303.8	0.589
159	318.2	0.596
161	321.8	0.597
171	339.8	0.603
181	357.8	0.623
190	374.0	0.640
193	379.4	0.630
220	428.0	0.618
230	446	0.655
240	464	0.681
250	482	0.682
250	482	0.698
280	536	0.759
287	548.6	0.765

by an arbitrary but very specific method which provides information of value and utility to the manufacturer of soaps and candles. Titers varying from 22 to 27.5° C. have been reported for soybean oil. Manufacturers generally offer soybean fatty acids for sale with a titer of 22 to 26° C.

Unsaponifiable matter: The unsaponifiable fraction of soybean oil includes all those substances which are not saponified by alkali but which are soluble in ether or petroleum ether. Included in this fraction are the sterols, some phytosterolines, hydrocarbons, alcohols, and ketones. The average content of unsaponifiable matter found in 153 samples of soybean oils examined at the Regional Soybean Industrial Products Laboratory was found to be 0.75 percent with individual variations ranging from 0.52 to 1.61 percent.

Standard specifications (312) for crude domestic soybean oil limit the maximum content of unsaponifiable matter to 1.5 percent and impose a penalty of 0.5 percent of the purchase price for each 0.5 percent in excess thereof.

Viscosity: Viscosity measurements have been reported for only a few soybean oils. They are given in various units and for different temperatures as shown in Table 14.

Table 14. Viscosity of Soybean Oils

Temperature	Units	Reference
°C.		
37.78	164.6 S.U.S.	Hufferd 1/
98.89	51.4 S.U.S.	Hufferd
148.89	38.9 S.U.S.	Hufferd
37.78	35.26 K V (Centistokes)	Hufferd
98.89	8.00 K V (Centistokes)	Hufferd
148.89	3.998 K V (Centistokes)	Hufferd
25.0 2/	51.0 Centipoises	Johnstone, Spoor, and Goss (211)
37.8 2/	31.5 Centipoises	Johnstone, Spoor, and Goss
50.0 2/	21.36 Centipoises	Johnstone, Spoor, and Goss
15.5	0.797 Poises	Inter. Crit. Table (311)
25	274 Redwood	Jamieson (206)
25	8.26-8.43 Engler	Jamieson

1/ Hufferd, R. W. Kendall Refining Company, private communication.
2/ Corrected figures. Viscosity data in reference are in error and must be multiplied by square of specific gravity to obtain correct values.

Miscellaneous data: In addition to the properties of soybean oils which have been discussed in the preceding pages there are several conversion factors derived directly or indirectly from these properties which are of considerable practical value. These factors are grouped here for convenient reference and are as follows:

Weight of soybean oil per gallon 7.67 pounds.
Weight of soybean oil per standard U. S. tank car approximately 61,000 to 62,000 pounds.
Volume of soybean oil per standard U. S. tank car approximately 8,000 to 8,060 gallons.

Fatty Acids and Glycerides

The percentage distribution of the fatty acids found in various petroleum ether-extracted soybean oils is shown in Table 15. (See Table 15 on page 48). In each case the compositions of the unsaturated acid fractions were calculated from the iodine and thiocyanogen numbers on the assumption that only oleic, linoleic, and linolenic acids were present in this fraction. However, Hilditch and Jasperson (178, 179) have found that normal soybean oils contain about 0.5 percent of hexadecenoic acid and it is not known to what extent this acid may be present in abnormal oils. The literature contains a number of reports of the analyses of soybean oils by other methods which have yielded results that are in substantial agreement with those given in Table 15. For example, Smith (400), Baughman and Jamieson (29), Jamieson, Baughman, and McKinney (207), Cruz and West (89), and Griffiths and Hilditch (139) employed the hexabromide number in determining the percentage of linolenic acid, whereas Hashi (165) and Hilditch and Jasperson (179) employed the fractionation of the methyl esters to determine the composition of a soybean oil. The last mentioned authors reported the presence of C_{14}-and C_{16}-unsaturated acids in these oils amounting to 0.64 and 1.44 percent, respectively, before deodorization, and to 0.53 and 1.60 percent, respectively, after deodorization.

The last seven samples of soybean oil referred to in Table 15 were prepared in the same manner under the same experimental conditions and were analyzed by identical methods throughout (29). Inspection of the data indicates that there is a remarkable constancy in the ratio of saturated to unsaturated acids of these soybean oils, which appears to be wholly independent of the iodine number of the oil from which the acids were derived. Of the seven soybean oils having iodine numbers ranging from 102.9 to 151.4, the saturated acids were found to comprise 12.7+0.8 percent and the unsaturated acids, 87.3 + 0.8 percent of the total acids present. It is also to be noted that within the limits of experimental error, the percentages of linoleic and linolenic acids increase more or less regularly with increasing iodine numbers of the oils, whereas the reverse is true of the oleic acid, which decreased progressively with the increase in iodine numbers of the oils.

Table 15. Composition of Solvent-Extracted Soybean Oils

Source	Variety	Oil			Fatty Acids 1/					Worker
		Iodine number	Thiocyanogen number	Unsaponifiable matter	Unsaturated	Saturated	Oleic	Linoleic	Linolenic	
				Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	
Manchuria	Unknown	137.05	80.5	1.2	84.6	15.3 2/	21.3	55.2	8.2	Kaufmann (222)
Manchuria 3/	Unknown	132.2	78.3	0.6	84.2	15.8 2/	25.9	51.2	7.1	Kaufmann
United States	Unknown	130.5	80.4	0.65	84.9	15.1 2/	24.7	53.7	6.5	Kaufmann
United States	Mammoth	134.2	81.9	0.57	85.2	14.8 2/	24.7	50.5	10.0	Kaufmann
Manchuria	Unknown	133.8	79.7	0.54	88.2	11.8	25.9	58.8	3.8	Kimura (225)
United States	Dunfield	102.9 5/	78.0	0.84	88.0	12.0 4/	60.0	25.0	2.9	Dollear, et al (103)
United States	Dunfield	127.3	80.2	0.66	86.9	13.1 4/	34.8	46.0	6.0	Dollear, et al
United States	Dunfield	124.0	79.6	0.70	86.8	13.2 4/	34.0	49.1	3.6	Dollear, et al
United States	Illini	131.6	81.3	0.84	88.0	12.7 4/	27.7	53.7	5.9	Dollear, et al
United States	Peking	137.8	84.5	0.93	87.6	12.4 4/	24.4	56.2	7.3	Dollear, et al
United States	Seneca	139.4	85.1	0.61	88.1	11.9 4/	24.7	55.4	8.0	Dollear, et al
United States	Wild Beans	151.4	87.4	2.20	86.5	13.5 4/	11.5	63.1	12.1	Dollear, et al

1/ Calculated on the basis of total fatty acids as 100 percent and not as glycerides.
2/ Bertram's oxidation method.
3/ Refined oil.
4/ Twitchell's lead salt alcohol method.
5/ Abnormal oil.

Although these data are limited with respect to the number of samples of soybeans which have been examined; nevertheless, in view of the randomness of their selection, they indicate that the distribution of the saturated and various unsaturated fatty acids in the soybean seed bears a definite relation to the iodine number of the oil, and their formation in these proportions appears to be governed by a specific and relatively invariable biochemical process, independent of varietal, climatic, or pedological conditions. These observations may be summarized as follows:

(1) The ratio of saturated to unsaturated acids in soybean oil is fairly constant irrespective of the total amount of oil present in the seed and of the iodine number of the extracted oil.

(2) The distribution of the unsaturated acids varies in a specific manner with the iodine number of the oil derived from the seed, but is independent of the total amount of acids which are formed during growth and maturation and stored by the seed in the form of various lipids.

Earlier workers including Smith (400), Baughman and Jamieson (29), Wallis and Burrows (443), Pfahler (347), Heiduschka and Eger (171), and others have reported values for the saturated fatty acids of soybean oil varying from 9 to 17 percent but this apparent variation results from the different methods of analyses which were applied rather than from actual differences in composition of the magnitude indicated by these figures. For example, the content of saturated fatty acids in soybean oils was found by Kaufmann (222) and by Earle and Milner (106) to be approximately 15 percent by the Bertram oxidation method, which value is 2 percent greater than that obtained by the Twitchell lead salt-alcohol method.

The content of myristic acid has been reported to vary from 0.6 to 1.07 percent, palmitic acid from 6.5 to 8.5 percent, stearic acid from about 3 to 6 percent, and arachidic acid from 0.2 to 0.7 percent.

According to Hilditch's rule of random distribution of the fatty acids among the glycerol molecules of seed-fats and liquid vegetable and animal fats, soybean oil should contain no fully saturated triglycerides. From a study of the composition of unhydrogenated and fully hydrogenated soybean oil, Hilditch and Jones (180) concluded that the original unhydrogenated oil contained, on a mole-percent basis, less than 1 mole-percent of fully saturated, 0 to 13 mole-percent of mono-unsaturated, 20 to 25 mole-percent of di-unsaturated, and 75 to 87 mole-percent of tri-unsaturated glycerides. The fully hydrogenated oil yielded 75 percent tristearin and 25 percent palmitoglycerides.

Previous to the work of Hilditch and Jones, Hashi (166) isolated oleodipalmitin from soybean oil by extraction with acetone. By brominating soybean oil and fractionating the resulting mixed bromides from petroleum ether, ethyl ether, and benzene, Suzuki (412, 415) isolated the following bromides: dilinoleolinolenin bromide, m.p. 78° C.; two linoleodilinolenin bromides melting at 117 to 118° C. and 158° C.; dilinoleolein bromide (oil); three oleodilinolenin bromides, m.p., 6°, 5°, and 3° C.; and linoleodiolein bromide, m.p. 3° C. Neither triolein nor trilinolein bromides were found. Hashi (167),

likewise attempted to separate the corresponding brominated glycerides from soybean oil by fractionation from various solvents. He obtained three bromides melting at 154°, 147°, and 74° to 75° C. and concluded from an examination of their properties that they consisted of linoleodilinolenin bromide, a mixture of oleodilinolenin bromide with dilinoleolinolenin bromide or with linoleodilinolenin bromide, and a mixture of trilinolein bromide with oleolinoleolinolenin bromide, respectively.

Sterols and Other Unsaponifiable Constituents

The unsaponifiable fraction from soybean oil amounting to from 0.5 to 2.2 percent has been incompletely investigated and except for the sterols, little is known concerning its composition. Klobb and Bloch (229) isolated a sterol, designated as soyasterol, from the unsaponifiable fraction of soybean oil which melted at 169° C. Matthes and Dahle (265) found the unsaponifiable matter of soybean oil (0.7 percent) to contain about 55 percent of crystalline materials and 45 percent of unsaturated oxygenated compounds. From the crystalline portion they separated two sterols, sitosterol and stigmasterol, melting at 139° and 169° C., respectively. Bonstedt (63) effected the separation of stigmasterol and sitosterol from the crude sterols derived from soybean oil by means of the Windaus and Hauth bromination method. Stigmasterol constituted 25 percent of the sterol fraction. The analysis of the hydrochloric acid addition product of sitosterol indicated the probable presence of dihydrositosterol, the separation of which was effected by fractional crystallization. The dihydrositosterol melted at 144° C. and had a rotation of $(\alpha)_D = +28°$. Repeated fractionation of the acetylated residue from the mother liquors gave an acetate melting at 143° C. and having a rotation of $(\alpha)_D = -46.8°$, which on saponification yielded a sterol melting at 142° C., and having a rotation of $(\alpha)_D = 44.8°$. This sterol was not identical with Anderson's γ-sitosterol. Bonstedt concluded that sitosterol from soybean oil evidently consisted of an almost inseparable mixture of several sterols which formed mixed crystals when fractionated from solvents.

Following the determination by Fernholz (120) of the constitution of stigmasterol from soybean oil, this sterol became an important source material for the preparation of various natural and synthetic hormones. Butenandt and coworkers (72, 74) prepared the corpus luteum hormone, progesterone, and related substances from stigmasterol and at practically the same time and by exactly the same methods Fernholz (121) reported the independent synthetic preparation of progesterone from this sterol. Shortly afterwards Butenandt and coworkers (71) also prepared dehydroisoandrosterone, the second male sex hormone isolated from urine, using both stigmasterol and cholesterol as raw materials.

Because of the interest in stigmasterol as a source material for the preparation of various sex hormones, the recovery of this sterol from soybean oil has attracted considerable attention. Although present in small amounts in crude soybean oil it may be obtained in fair yields by bromination and fractional crystallization, according to the method of Windaus and Hauth, (464,465),

of the mixed sterol acetates derived from the unsaponifiable fraction of this oil. Detailed directions for the preparation of stigmasterol from 1000 kilo quantities of soybean oil are given by Steiger and Reichstein (404).

The commercial recovery of crude sterols from soybean oil dates from the earliest application of continuous solvent extraction of soybeans in Germany. One of the early processes for the continuous extractions of soybeans employed the method of countercurrent washing of the oil with cold alcohol to remove free fatty acids and produce a neutral oil (40). During the recovery of the alcohol by distillation the crude mixed sterols concentrated in the residue and often crystallized in the still.

Holtz (188) mentions the fact that sterols are found in the phosphatidic sludge from solvent-extracted soybeans but points out that acidulated soapstock obtained during alkali refining is a much better source of these substances. Holtz found that practically all of the sterols present in solvent-extracted soybean oil accumulates in the soapstock during alkali refining where it forms 2 to 3 percent of the total. Ten kilograms of soapstock was found to yield 185 grams of mixed crystalline sterols melting at 138-139° C. Advantage has been taken of this fact to produce crude soybean sterols from so-called still pitch which remains on distillation of the fatty acids recovered by acidulation of refinery soapstock.

The recovery of crude sterols from the alcoholic extract of soybean oil, the acetone extract of soybean lecithin, and from the steam condensate from the deodorization of soybean oil has been described in a patent by Schwieger (388). Crude sterols can also be recovered from soybean oil by molecular or short-path distillation (351, 431).

Wolff (467) has called attention to the loss of sterols and hydrocarbons on filtration and neutralization of soybean oil.

Kraybill, Thornton, and Eldridge (240) have described a method of obtaining crude mixed sterols by adsorbing them on aluminum silicate (236--239) during passage of the oil through a packed tower, or by mechanical agitation of the oil and adsorbent, followed by extraction of the adsorbent with acetone to recover the adsorbed sterols.

Besides the sterols mentioned above the only other substances isolated from the unsaponifiable fraction of soybean oil have been a ketone and an unsaturated hydrocarbon. Schmalfuss and Treu (383) isolated methyl-n-nonyl ketone by steam distilling saponified soybean oil and extracting the condensed distillate with ether. The ketone was identified by preparation of the corresponding semicarbazone.

Nakamiya (302) isolated an unsaturated hydrocarbon, $C_{18}H_{32}$, from the unsaponifiable fraction of soybean oil. The same hydrocarbon was previously isolated from fish liver oil and named gadusene. On hydrogenation the hydrocarbon gave gadusane, $C_{18}H_{38}$, b.p. 85 to 86° C., $n_D^{20} = 1.4632$.

Oil-Soluble Pigments

The pigments present in soybean oil have been incompletely characterized, and very little is directly known relative to the nature or the amount of these substances which are present in various types of soybean oils. On the basis of the spectral transmittance curves of many samples of crude and refined cottonseed oils, as well as a smaller number of soybean oils, McNicholas (259, 260) concluded that these oils contained various amounts of green (chlorophyll), reddish yellow (carotenoid), and brown pigments. The work of McNicholas has been substantiated and extended by Bickford, Anderson,and Markley (34) who examined a variety of crude, semirefined, and completely refined soybean oils with respect to their absorption spectra as has been previously mentioned under the subject of the color of soybean oils.

Considerable effort has been directed toward the measurement of the color of soybean oil in terms of various color scales (144, 350, 430); studies relative to the effect of chemical and physical reagents on color values have been carried out (448); and technological processes for the reduction or removal of color have been devised (107, 313, 314). However, very little effort appears to have been made to isolate and identify the various pigments which are present in the oil and which are responsible for its color.

As a result of considerable interest in the vitamin A content of soybeans a few quantitative determinations have been made of the carotenoid content of the seeds. For example, Lantzing and Van Veen (244) identified the carotenoid pigments of soybeans grown in the Netherlands Indies as β-carotene and xanthophyll. The total carotenoid content of the varieties which they examined were found to vary from 70 to 180 international units and the β-carotene portion from 70 to 130 international units per 100 grams of ground soybeans. Sherman and Salmon (395) found that soybeans in the green stage contained 212 to 705 γ per 100 grams of fresh material while mature beans contained 18 to 243 γ per 100 grams. They also concluded that the principal carotenoid pigment in the soybean extract was β-carotene and that if crytoxanthin was present at all it occurred in negligible amounts. In a later publication Sherman (393) reported that the carotenoid pigments extracted from mature seed of five varieties of soybeans consisted of 80 to 88.8 percent β-carotene and 2.5 to 11.8 percent α-carotene.

In a series of 4 publications on the unsaponifiable fraction of soybean oil Nakamura,and Tomita (304, 305, 306, 307) claim to have isolated from this oil a fucoxanthin-like pigment, taraxanthin, lutein, cloxanthin-like and several other carotenoid pigments.

The type and amount of pigments present in crude soybean oils vary markedly with the variety of beans and the method of producing the oil. A high moisture content and the use of certain solvents lead to an increase in the amount of chlorophyll extracted, whereas a low moisture content and the use of other solvents repress the removal of chlorophyll. The conditions of pressing, likewise, markedly affect the color of the oil, e.g., cold-pressed

oils are generally lighter in color than hot-pressed oils. With increasingly high temperatures of the expeller barrel, the expressed oils become darker red and in extreme cases, brown or nearly black (13). Part of this increased color is probably caused by the removal of additional colored substances with increased pressure, but a greater part is undoubtedly due to the production of decomposition products from the thermally unstable pigments, carbohydrates, and other materials present in the seed.

During the process of alkali refining and subsequently during bleaching with various activated carbons and earths, the color of soybean oil is markedly reduced. The processes of deodorization and hydrogenation likewise affect the color but to a lesser degree. According to McNicholas (259) the green pigments are the most fugitive to the bleaching action of light and chemicals, and are nearly completely removed by treatment with fuller's earth or exposure to sunlight. Commercial bleaching results in considerable reduction in the brown and red pigmentation, whereas the yellow pigments are the most resistant to complete removal. It is possible, however, to produce nearly colorless soybean oil by laboratory refining and bleaching with certain types of adsorbents. The effects of alkali refining, bleaching, deodorization, and winterization on the reduction of the color of soybean oil have been reported by Bickford, Anderson, and Markley (34). Based on the examination of the spectral transmittance curves of 84 samples of soybean oils representing various types of processing and different refining procedures they concluded that: (1) Chlorophyll, which is present in various concentrations in crude soybean oil, is completely absent from the finished oil so far as spectrophotometric evidence is concerned, and (2) the concentration of carotenoid pigments is greatly reduced during refining operations with the greatest loss occurring during deodorization.

Antioxidants

It has long been recognized that soybeans and crude soybean oil contain antioxygenic bodies which are effective inhibitors of oxidative rancidity. The identity and chemical configuration of the substances which are responsible for this protective action are at present unknown; however, various products, including the meal and flour, pigments, sterols, phosphatides, and even refined hydrogenated soybean oil itself have been claimed to be the actual inhibitor or at least to act as inhibitors to rancidity.

Green and Hilditch (135) found that if solvent-extracted soybean meal was first digested with dilute solutions of organic acids, e.g., 2 percent acetic acid in water or acetone, and then heated with methyl alcohol it yielded about 10 percent of soluble material. It was found that part of this methyl alcohol-soluble material was soluble in cold acetone to the extent of about 2 percent on the basis of the original meal and that it consisted of a viscous gum having marked antioxygenic properties. When 0.2 percent of this product was added to the distilled esters of olive oil their stability at 97.5° C. was increased 4 to 8 times. In a subsequent publication by Hilditch

and Paul (182) evidence was advanced to indicate that the antioxygenic substance was a basic oxygenic compound rather than a basic nitrogeneous compound but its exact nature could not be determined.

Musher (288, 289, 290) claims that soybean flour or meal, either untreated, or after bleaching, or after solvent extraction, when added to lard or other fatty products imparts to them protection against rancidification. Musher (291, 292, 293) further claims that the addition of 0.1 to 0.5 percent of a concentrated water extract or an alcoholic extract of soybean flour which has previously been extracted by fat solvents when added to lard and other food products will prevent or retard rancidification.

Dahle and Nelson (92) attempted to determine the active fraction of oat and and soybean flours with only partial success. The phospholipid fraction and the alcoholic extract exhibited greater antioxygenic properties when added to dry fresh milk fat than did either the aqueous acetone, ether, or hexane extracts. In every case the oat flour extracts appeared to have greater protective powers in dry fresh milk fat than did the corresponding extracts of soybean flour.

Grettie (137) claims in a patent that the use of one to ten percent of crude soybean oil as such or after hydrogenation when added to lard or other shortening will stabilize it with regard to rancidification. In further patents (138, 195, 280) the use of hydrogenated refined soybean oil is claimed to inhibit rancidity in shortening, lard, oleo oil, and products containing these substances.

Carotenes which are known to be present in soybean oil have been claimed by some investigators to be antioxidants while other equally competent investigators have found them to be prooxidants. Franke (124) observed that carotene and related compounds catalyzed the autoxidation of linoleic acid to about the same extent as did ergosterol. All of the carotenoids including vitamin A, carotene, lycopene, xanthophyll, and zeaxanthin were found by Franke (125) to function as positive catalysts toward free fatty acids, e.g., oleic and linoleic, but were without effect on their glycerides, e.g., olive oil, linseed oil, and cod-liver oil. Monaghan and Schmitt (285) found that carotene greatly inhibited the oxidation of linoelic acid, but oxidized carotene slightly accelerated this process. Newton (317) on the other hand found that carotenes in the form of concentrates, or some substance associated with them in nature, acted as antioxidants for certain fats. High temperature treatment of the fat to bleach it increased the antioxidant effect. According to French, Olcott, and Mattill (126) purified carotene from any source slightly reduced the induction period of fats and increased the rate of subsequent oxidation.

Nakamura and Tomita (310) observed very small stability indices, 0.95 to 1.41, with 6 different carotenoid pigment fractions which were added to refined soybean oil in concentrations of 0.05 percent. One fraction was considered as a prooxidant, and one (lutein) as a strong antioxidant. The other four fractions were considered as weak antioxidants.

The conflicting observations concerning the effect of carotenoid pigments on the stability of vegetable oils can be explained on several grounds, namely the purity of the preparations used and the presence or absence of light during the experimental work. Bradway and Mattill (65) found that purified carotene actually functioned as a prooxidant to shorten the induction period of autoxidizable fats, whereas crude carotene or carotene concentrates by virtue of associated inhibitols acted as antioxidants.

Franke (124) found that chlorophyll and chlorophyllides had little or no effect on the autoxidation of linoleic acid, presumably in the absence of light. Meyer (274, 275) investigated the effect of the presence of chlorophyll and other pigments on the photosensitized oxidation of a considerable number of unsaturated compounds including oleic acid, undecenic acid, and ergosterol. In the photochemical oxidation of oleic acid, some terpenes and ergosterol, it was found that chlorophyll was an active catalyst and was even more effective than eosin. With chlorophyll as the catalyst, oleic acid and ergosterol took up 0.5 mol of oxygen to form hydroxyoleic acid, in the case of oleic acid, without the formation of any detectable peroxides.

From the work of Franke, Meyer, and of Bickford, Anderson, and Markley (35) and others, it may be concluded that any natural pigment such as the carotenoids, chlorophyll, or its colored derivatives, or any fluorescent dye contained in or added to a highly refined autoxidizable oil will act as a prooxidant in the light, but will be inactive, or act as an antioxidant in the dark. These conclusions follow from the fact that the pigments mentioned above are capable of absorbing appreciable amounts of photochemical energy in the form of light, which energy may be transferred to and result in activation of other molecules present in the oil thus enabling them to take part in oxidation reactions. On the other hand, in the absence of light, many of these pigments being themselves readily susceptible to oxidation, absorb oxygen and thereby protect other readily oxidized constituents of the oil.

In a similar manner the naturally occurring sterols of soybean oil have been considered to be antioxidants by some investigators, and to be wholly inactive by others. The only sterols known to be present in soybean oil are stigmasterol, sitosterols, and dihydrositosterol. Evans (116), using the peroxide index of cottonseed oil catalyzed by cobaltic oleate at room temperature, found that cholesterol and stigmasterol had no antioxygenic activity but that sitosterol afforded considerable antioxidant protection.

Franke found cholesterol to have little or no effect on the autoxidation of linoleic acid while ergosterol was found to be active under the same conditions. Royce (375) demonstrated by several different methods that the mixed sterols from corn oil and cottonseed oil had no antioxidant activity in finished cottonseed oil.

Mattill and Crawford (267) attributed the observed antioxygenic effect of certain types of corn oil to the presence of sterols but later Mattill (266) concluded that purified cholesterol and sitosterol were ineffective as antioxygens and that the protection against oxidation observed with these substances was due to some antioxygenic constitutent accompanying the sterols.

Roller (374) found that the antioxidant activity of wheat germ oil for fats and oils was independent of the sitosterol content of the germ oil.

Nakamura and Tomita (308, 309) found that although the unsaponifiable fraction prepared by several different methods possessed antioxidant activity when added to refined soybean oil, this was not observed to be the case with the sterols isolated from the same fractions. The crystalline sterol fraction melting at 139.5° and having a rotation $\alpha_D^{20} = -34°$ in chloroform solution was added to refined soybean oil in concentrations varying from 0.1 to 1.0 percent. Comparison of the treated and untreated soybean oil showed no difference in stability.

Experiments at the Regional Soybean Industrial Products Laboratory with the purified mixed sterols from soybean oil and the corresponding pure sitosterols and stigmasterol indicated that they afforded refined soybean oil no antioxidant protection under the conditions obtaining in the Swift accelerated stability test.

The sterols which are present in soybean oil are quite stable products and relatively unreactive even in the isolated state and it is not to be expected that they should be any more reactive in an oil system. It may be concluded, therefore, that the sterols present in soybean oil do not function as antioxidants under the conditions pertaining in the crude or refined oil.

Olcott and Emerson (333) have shown that the tocopherols are effective for lard. Thaler and Schulte (425) investigated the effect of pure d, l-α-tocopherol and acetate on the aldehyde and ketone rancidity when added to ethyl oleate and peach kernel oil in concentrations of 0.1 and 0.5 percent. Antioxidant activity was observed with the tocopherol as well as with the acetate. Isler (196) found that α-tocopherol was autoxidized in a Warburg-Apparatus at 38° C. and, therefore, it may be presumed to have antioxygenic activity. However, tocopherols have never been isolated from soybean oil and in view of the low vitamin E activity of this oil it is unlikely that it contains any appreciable amount of tocopherols.

Bollmann (41, 42) was perhaps the first to claim that phosphatides in general and commercial soybean lecithin in particular when added in amounts of 0.05 to 1.0 percent to refined edible oils prevented or retarded oxidative rancidity in these products. From time to time other patents have appeared which claim that the addition of soybean phosphatides in various amounts to such products as shortening, lard, bakery goods, gasoline, mineral lubricants, etc., will afford protection against oxidation changes. Many similar claims have been made in general articles relating to the use of phosphatides (110, 461, 474).

According to many patents the antioxidant activity of soybean phosphatides containing added ingredients is greater than that of the phosphatides alone. Mitchell (279) claims that the addition of one to ten percent of hydrogenated refined soybean oil and 0.05 to 0.2 percent of lecithin to an oil affords marked antioxidant protection compared with the addition of either substance singly. Musher (294, 295, 296, 297, 298) claims that a mixture of carbohydrates and phosphatide affords superior antioxidant protection to lard and other fats.

Thurman (427, 428, 429) claims that corn oil and cottonseed oil phosphatides are superior to soybean oil phosphatides as inhibitors because the former contain acids of a lower degree of unsaturation than the latter. This claim is predicated on the fact that soybean phosphatides contain as part of the molecule, linolenic acid, which is readily susceptible to oxidation with the formation of off-flavors and -odors. A similar claim is made by Engelmann (112) who patented the stabilization of aqueous emulsions of soybean phosphatides by the addition of peroxides.

In another patent (195) it is claimed that shortening can be stabilized against rancidity by simply mixing it with one to ten percent hydrogenated refined soybean oil. It is claimed in this patent that deodorization at a temperature sufficiently high to destroy the lecithin does not affect the antioxidant value.

Royce (376) claims that synthetic phosphatides in which one hydroxyl group of the glycerol molecule is unesterified are superior antioxidants to the natural product. The antioxygenic activity is attributed to the free hydroxyl group of the synthetic product. Shappirio (391) assumes that the antioxygenic portion of the molecule is betaine or some related trimethylamine derivative, and hence he claims their use or the use of the corresponding synthetic alkylamino-aklyl-phosphoric acid esters as antioxidants. Eckey (108, 109) and Richardson (369, 370) have patented the use of phosphoric acid and related substances as antioxidants for the stabilization of fats and oils.

From the above cursory summary of the patent literature it would appear that the phosphatides themselves as well as all of the component parts of the phosphatide molecule have been claimed to be responsible for the antioxidant activity of these substances.

On the experimental side Kockenderfer and Smith (231) reported that two commercial samples of soybean lecithin which they examined were found to be weak antioxidants having indexes of 1.7 and 1.8, respectively, when added to fats in concentrations of 0.1 to 0.2 percent. The antioxygenic index was defined as the induction period of the fat with added antioxidant divided by the induction period of the fat alone. Based on oven tests at 70° C. Royce (375) obtained somewhat similar results. The addition of 0.2 percent of vegetable lecithin to finished cottonseed oil gave an antioxidant index of 2.0, whereas addition of 0.1 percent of the same product to hydrogenated shortening gave an index of 1.5.

Roller (374), and Triebold, Webb, and Rudy (433) reported that lecithin did not lengthen the induction period for oils. The latter authors found that choline derived from lecithin also failed to lengthen the induction period.

Evans (116), using the peroxide index of a cobalt oleate catalyzed cottonseed oil at room temperature, found vegetable lecithin to have relatively high antioxidant value. However, under the same experimental conditions sitosterol which is known beyond doubt to be inactive in this respect was also found to have appreciable antioxidant value. Furthermore, the procedure by Evans, namely, the addition of the very active oxidation catalyst, cobalt oleate, to the test samples and admission of pure oxygen to the oil once or twice each day provides no information concerning the probable behavior of an oil under normal conditions of storage or use. Evans concluded that the antioxidant activity of vegetable lecithin probably resided in the free amino or hydroxyl group of lecithin or cephalin. However, he also concluded that the antioxidant property of vegetable lecithin is easily destroyed by heating to 65° C., which latter conclusion is not compatible with the former, since a temperature of 65° C. cannot have an appreciable effect on amino or hydroxyl groups. Also this temperature is below that prevailing in the various processing steps followed in the production of vegetable lecithin. An antioxidant having protective action only at temperatures below 65° C. would be limited in its application in the field of fats and oils.

Holmes, Corbet, and Hartzler (186) found that hydroquinone and commerical soybean lecithin afforded protection against the destruction of vitamin A in halibut and cod-liver oils. Hydroquinone was found to be more active than the commercial lecithin even in much smaller concentrations but the combination of the two was found to be more active than either one separately. The results obtained with vitamin A do not appear to be comparable with those ordinarily observed with autoxidizable oils, since no induction period is apparent either with or without added antioxidants. In the case of the lecithin experiments the destruction of vitamin A was found to be a straight line function of time and therefore does not correspond to an autocatalyzed reaction such as occurs in the rancidification of oils. The two reactions, the destruction of vitamin A and autoxidative rancidification, appear therefore to be unrelated phenomena.

Olcott and Mattill (334, 335) found that commercial preparations of soybean lecithin had moderate antioxygenic action on refined cottonseed oil (antioxidant index 2 to 4 for 0.1 percent lecithin), little effect on lard (antioxidant index 1.3 to 1.6 for 0.2 percent lecithin), and none at all on mixtures of lard and cod-liver oil. Purified lecithin was found to have no antioxygenic action while purified cephalin possessed some activity. No quantitative relation was found between the amount of purified cephalin used and the prolongation of the induction period. They concluded that the monobasic phosphoric acid radical of cephalin was probably responsible for the observed antioxygenic action.

Hilditch and Paul (182) found that carefully purified soybean phosphatides containing phosphorus and nitrogen in almost exactly the proportions demanded by a diacid-glycerophosphoric ester of choline were completely lacking in antioxygenic activity when added to the distilled esters of olive oil fatty acids.

Nakamura and Tomita (303) using highly purified lecithin and cephalin from solvent-extracted soybean oil found them to be either inactive or weak antioxidants for refined soybean oil when used in concentrations varying from 0.20 to 1.06 percent. The antioxidant index was found to vary from 1.05 to 1.17 and no relation was observed between the concentration and degree of activity of these substances in soybean oil.

Diemair, Strohecker, and Reuland (102) determined the antioxidant activity of lecithin and its components including glycerol, oleic, and linoleic acids, glycerophosphoric acid, choline, and colamine. Commercial soybean lecithin purified by a single precipitation from petroleum ether by addition of acetone was added to olive oil in concentrations of 0.5, 1.2, and 4 percent. In the case of the Kreis-Täufel reaction no difference was observed between the treated and untreated samples but other tests indicated that concentrations of 0.5 to 2.0 percent were moderately effective, and concentrations of 2.0 to 4.0 percent quite effective. The authors consider the latter concentrations impractical and most patent claims specify 1.0 percent as the maximum addition required for effective protection against rancidification.

Choline, in concentrations of 0.7, 1.25, and 5.0 percent, was found to be an effective inhibitor but it was stated the concentrations used were considerably greater than that corresponding to its occurrence in lecithin, and its use was followed in a short time by the development of a fishy odor resulting from the production of trimethylamine. The authors state that colamine, a component of cephalin, appears to be useless as an antioxidant. None of the other components of lecithin exhibited antioxidant activity.

Franke (124) found that lecithin and acetylcholine actually acted as catalysts in the autoxidation of linoleic acid.

From a theoretical point of view it is not to be expected that pure lecithin should exhibit antioxygenic activity. Mattill and coworkers (146, 266, 332, 336) found that the antioxygenic substances both natural and synthetic, comprised three general classes or types of substances: (1) acid-type inhibitors, (2) inhibitols and hydroquinone, and (3) phenols. These substances are characterized by the presence of polyhydroxy groups, polycarboxylic groups, hydroxy-carboxylic groups, or hydroxy groups in close proximity to ethenoid linkages.

If lecithin is assumed to contain a betaine linkage none of these conditions exist in the lecithin molecule, whereas if a straight chain configuration is assumed for the phosphoric acid ester portion of the molecule it would contain two free hydroxyl groups. On the basis of the best available experimental evidence it would appear that lecithin possesses a betaine or endo-salt structure (140, 141).

Delezenne and Fourneau (99) claim to have proved that lysolecithin has an anhydride formula and Grün and Limpächer (140, 141) likewise claim to have established this structure for synthetic lecithin. Grün states that without doubt the phosphatides isolated from natural products are anhydride endo-salts, at least when completely dried. As further evidence he states that the pure phosphatides react neutral and that the strong basic hydroxyl group of choline and the acidic hydroxyl radical of phosphoric acid cannot coexist in the same molecule.

If the natural pigments, sterols, and phosphatides are eliminated as possible sources of the antioxygenic activity which has been observed to reside in soybeans and crude soybean oil, what other substance or substances may be considered as the cause of this activity?

Olcott and Mattill (335) found that the unsaponifiable fraction of many vegetable oils contains compounds which are active antioxidants for lard and designated these unknown substances as inhibitols. They found that these inhibitols could be concentrated by processes of solvent extraction, saponification and extraction, fractionation to remove the carotenoid pigments and sterols, and finally recovering the inhibitol by fractional distillation within a temperature range of 90 to 220° C. at 0.002 to 0.2 mm. pressure (268). These inhibitols were found to be transparent oils which resisted crystallization. They contained at least one phenolic hydroxyl group and one double bond. Reagents which attacked the hydroxyl group or saturated the double bond were found to inactivate the inhibitol. The activity could, however, be regenerated by reestablishing the hydroxyl group and the double bond. The double bond was found to be resistant to hydrogenation but readily added chlorine and bromine. These inhibitols exhibited a strong absorption at 2940 Å which was roughly proportional to their antioxidant activity.

Similar inhibitol fractions were observed at the Regional Soybean Industrial Products Laboratory to collect in the first fractions from crude, and to a less extent from refined, soybean oils when these oils were submitted to short-path or molecular distillation (33). The temperature of distillation was considerably below that at which the glycerides distilled, which in turn was below the temperature at which the phosphatides distilled. In fact, analyses of the distillates and undistilled residues for phosphorus indicated that the phosphatides gradually accumulated in the undistilled portions of the oil. The first distillates were found to contain sterols, carotenoid pigments, and a light colored oil similar to that described by Mattill and Olcott. The sterols and the light colored oil were separated by solvent fractionation and the original distillate, sterols, and oily residue were separately examined for antioxidant activity. The sterol fraction was found to be inactive, whereas, the original distillate and residual oil possessed marked antioxidant properties when added to refined, edible grade, soybean oil. Attempts to crystallize the oil or prepare crystalline derivatives were unsuccessful. A colorless amorphous solid was obtained by reacting the crude product with cyanic acid gas. The product was not identical with the allophanates of the natural tocopherols.

Green and Hilditch (135) reported previously unpublished work carried out by H. K. Dean describing the preparation of an orange-colored syrup from the unsaponifiable fraction of linseed oil which possessed strong antioxygenic activity. The syrupy oil was found to reduce Tollens' reagent and alkaline permanganate and readily absorbed bromine. It had an iodine number of 145 and possessed absorption bands at 2560, 4200, 4450, and 4750 Å, the latter three bands being attributed to the presence of carotenoids. Green and Hilditch obtained a viscous, gummy product by extraction of soybean cake, which although it apparently contained other substances resembled the oily, antioxygenic product described by Mattill and Olcott. They, like previous workers, were unable to isolate and identify the pure antioxidant present in the viscous oil.

Despite efforts to completely characterize the oily fraction obtained by Mattill and Olcott and other workers, it would appear that this substance either is or contains a constituent which is responsible for the antioxidant activity observed in soybeans and crude soybean oil. It is also quite probable that the antioxidant activity observed by various investigators with products such as unsaponifiable matter, sterols, carotene, and commercial phosphatides, results from the presence of this oily constituent in the semi-pure products used in their experiments. It also explains the fact that these products when rigorously purified are found to be inactive, or in some cases accelerators of rancidity and autoxidation of fats and oils.

Phosphatides

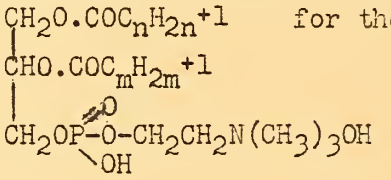
Soybean oil contains a variable mixture of glycerophosphoric acid esters which are collectively designated as phosphatides, or according to Chibnall and Channon (80, 81, 82, 83) as phosphatidic acid esters. The apparent content of phosphatides of the oil varies with the method by which the oil is removed from the seed but generally ranges from 1.5 to 2.5 percent.

As has been previously mentioned phosphorus is present in the soybean seed in the form of phytins, phosphatides, nucleic compounds (phosphoproteins), and inorganic salts having different and varying solubilities in different solvents and under various conditions of extraction. The exact quantitative determination of the content of phosphatides in the seed is therefore extremely difficult as has been pointed out by Earle and Milner (105) and much confusion has been created by investigators who have been unaware or ignored the difficulties of the problem (111, 187, 205, 245, 352, 355). Only a small fraction of the phosphorus present in the seed is in the form of phosphatides but practically all of the phosphorus of the oil appears to be present in this form.

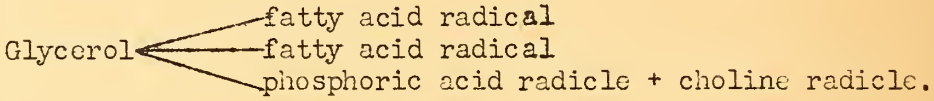
Soybean oil contains both lecithins and cephalins, the former being present to the extent of about 30 to 35 percent of the total phosphatides.

Lecithins are distinguishable from the cephalins by their ready solubility in alcohol and ether in contrast to the lesser solubility or complete lack of solubility of the latter in alcohol.

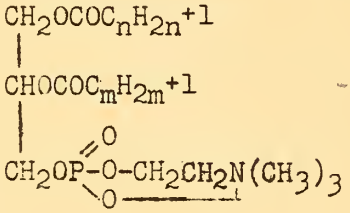
Since lecithins yield on hydrolysis two molecules of fatty acid, one molecule of phosphoric acid, and one molecule of choline, Strecker (217) proposed the general formula,



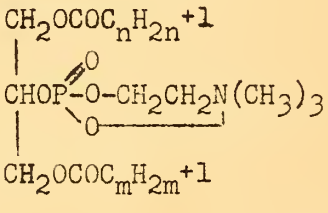
for these compounds, or more simply,



Unsaturated acid radicals may be substituted for one or both of the saturated fatty acids and the phosphoric acid radical may occupy either the α- or β-position in the glycerol molecule. If it is presumed that the choline radical is present in a betaine linkage, the naturally occurring lecithins are best represented by the formulas shown below.



α- Lecithins



β- Lecithins

If colamine (hydroxyethylamine, $CH_2NH_2CH_2OH$) is substituted for choline in the above formulas, a similar series of α- and β-cephalins is obtained. It is obvious that by varying the fatty acid radicals of the lecithin and cephalin series a very considerable number of isomeric and homologous phosphatides are possible. In fact Suzuki and Yokoyama have calculated that 70 isomeric and homologous lecithins are theoretically possible with four different fatty acids. 7/

Prior to the work of Levene and Rolf (250, 251, 252) on soybean phosphatides, plant phosphatides were usually supposed to be varieties of lecithin containing palmitic, stearic, and oleic acid components. These workers showed

7/ For a general treatment of the chemistry, physiology, technology, and uses of phosphatides, especially those derived from soybeans the reader should consult the chapter on phosphatides prepared by Ad. Grün which appears in the first volume of Hefter-Schönfeld's Chemie und Technologie der Fette und Fettprodukte (385) and also the chapter by E. H. Winterstein and A. Winterstein on phosphatides in Klein's Handbuch der Pflanzenanalyse (227).

that the phosphatides present in the soybean consisted of a mixture of lecithins and cephalins containing palmitic, stearic, oleic, linolenic, and linoleic acids. They prepared optically-active barium glycerophosphates from the lecithin and cephalin fractions and concluded, therefore, that the phosphoric acid was attached to an α-position in the glycerol molecule. Webster (449) recorded Hanus iodine values of the mixed fatty acids present in the acetone-insoluble phosphatides as 92.5, this indicating the presence of acids more unsaturated than oleic.

Suzuki and Nishimoto (414) separated a series of cephalins from soybeans which yielded on hydrolysis only β-glycerophosphoric acid and fatty acids. The fatty acids consisted of about 50 percent stearic acid and a mixture of approximately equal parts of linoleic and linolenic acids. From the brominated cephalin they obtained at least two forms of linoleostearo-β-cephalin and linolenostearo-β-cephalin.

Suzuki and Yokoyama (416) separated from soybeans α- and β-lecithins on the basis of their differences in solubility in warm acetone, the β-forms being much more soluble. By hydrolysis of the cadmium chloride salt of lecithin they obtained the barium glycerophosphate equivalent to 35 percent of the β-acid. Hydrolysis of the α- and β-forms gave 24.3 and 20.1 percent of palmitic acid, 32.4 and 53.8 percent of dibromostearic acid, 42.2 and 24.8 percent of tetrabromostearic acid, and 1.1 and 1.4 percent of hexabromostearic acid, respectively. Similarly Yokoyama and Suzuki (475) isolated from soybeans a series of α-lecithins as the double salts of cadmium chloride which, on bromination and fractionation by means of ethyl ether and chloroform, gave four bromides. Three of the bromides, palmitolinoleo-α-lecithin, oleolinoleo-α- lecithin, and dioleo-α-lecithin, were oils and the fourth a solid, dilinoleo-α-lecithin, melting at 113 to 114° C. The structures were established by hydrolysis and identification of the fatty acids.

Hilditch and Pedelty (183) examined the component acids present in the alcohol-insoluble and alcohol-soluble fractions of phosphatides derived from soybeans. The fatty acid distribution of the phosphatide fractions is shown in Table 16 on page 64. From these results it is evident that the phosphatides contain all the characteristic fatty acids which are present in the glycerides. The proportion of saturated acids is higher in the phosphatides than in the glycerides. It is also evident that linoleic acid is the chief acid of both the phosphatides and glycerides derived from soybeans.

Belozerskii and Kornov (31) found that the embryos of soybeans contained 3.15 percent of phosphatides and the cotyledons, 2.1 percent. Both cephalins and lecithins were isolated, the latter of which yielded on hydrolysis oleic, linoleic, and linolenic acids. Linoleic and linolenic acid were found to be present in greater amounts in the cotyledon-lecithin than in the embryo-lecithin.

Recently McKinney, Jamieson, and Holton (258) have again raised the question of the probable occurrence of phosphatides in the form of glycosidal complexes. Rewald (356, 365, 368) has shown, however, that although phosphatides are closely associated with carbohydrates, the union is not a chemical one, since extraction with the proper solvent and other physical treatments

Table 16. Component Acids in Phosphatides Derived from Soybeans

Acid	Alcohol-insoluble		Alcohol-soluble	
	Weight-percent	Mole-percent	Weight-percent	Mole-percent
Palmitic	11.7	13.1	17.3	18.6
Stearic	4.0	4.0	--	--
Arachidic	1.4	1.3	--	--
Hexadecenoic	8.6	9.6	5.5	6.0
Oleic	5.5	5.7	19.0	18.5
Linoleic	63.3 1/	61.5	53.0	52.0
Linolenic	--	--	3.7	3.6
C_{20} unsaturated	5.5	4.8	1.5	1.3

1/ Includes small amount of linolenic acid, not exceeding 4 percent.

are sufficient to separate the phosphatides in relatively pure form from carbohydrates or fats. Soybean phosphatides can be freed of carbohydrates according to Rewald (367) simply by suspending them in water and shaking the suspension with trichlorethylene. The phosphatides go into the trichlorethylene phase while the carbohydrates remain in the water phase. In this connection it might be mentioned that Klenk and Sakai (228) isolated from the cephalin fraction of purified soybean phosphatides after hydrolysis with hydrochloric acid, inositol, glycerophosphoric acid, and the brucine salt of inositolmonophosphoric acid melting at 236° C. Other phosphoric acid esters were found to be present.

It is to be expected from a consideration of the nature of the carbohydrate materials present in soybeans, and of the properties and method of removal of the phosphatides, that the two should form difficultly separable mixtures. The carbohydrates are present in soybeans both as free sugars and as glycosides, e.g., saponins, phytosterolines and isoflavone glycosides. The relative solubility and emulsifying properties of the various carbohydrate

materials, lecithins, and cephalins in water, alcohol, acetone, and other organic solvents are such that the preparation of carbohydrate-free phosphatides is extremely difficult. Much the same solubility relations hold for the non-phosphatidic nitrogenous constituents which are associated with the phosphatides. In fact Hofer (185) has prepared synthetic lecithin-protein complexes, e.g., serum-albumin-lecithin complex which could not be broken up by ether extraction but which was readily soluble and could not be filtered. If ether and alcohol were added to a lecithin solution at pH 2.5 the lecithin was found to pass quantitatively into the ether and separate again at pH 7.0 when one percent of potassium chloride was added to the solution.

Properties, preparation, and uses of phosphatides: The preparation and uses of phosphatides are inseparably associated with the physical and chemical properties of these substances and it is therefore advantageous to discuss these three subjects simultaneously. The production and purification of the commercial product is, however, deferred to the section on processing.

It is to be understood that by phosphatides is meant the mixture of lecithins and cephalins which are generally isolated from soybeans or soybean oil in the laboratory. Commercial preparations of phosphatides often referred to as vegetable lecithin, are stated to consist of a mixture of approximately 60 percent phosphatides, 35 to 40 percent of soybean or other oil, and 4 to 6 percent moisture. However, these preparations also contain various amounts of sterols, sterol esters, carbohydrates, glycosides, pigments, and many other minor lipid and non-lipid constituents.

The commercial product is usually a yellowish-brown, waxy solid at room temperature which is soluble in ether, petroleum ether, benzene, and fatty oils, partially soluble in alcohol, and relatively insoluble in acetone and ethyl and methyl acetates.

The relatively oil-free preparation of soybean phosphatides ordinarily obtained by laboratory preparation, or commercially by special refining, is a yellow wax-like mass. When entirely freed of oil it rapidly undergoes oxidation on exposure to air and turns black, whereupon it loses its solubility in alcohol and ether and becomes somewhat soluble in water. Soybean phosphatides readily form addition compounds with the salts of heavy metals such as cadmium chloride and advantage is often taken of this property as a means of fractionation and purification of the various lecithins and cephalins present in the mixture. They are readily saponified by strong alkalies and hydrolyzed by strong acids.

Soybean phosphatides are highly hygroscopic, swell in water, and form colloidal solutions, and generally behave as colloids. One of their most important properties is their effectiveness in lowering the surface tension of aqueous solutions. Lecithin forms monomolecular films and the interfacial tension for the system benzene-water containing lecithin has been measured at various pressures by Alexander and Teorell (4).

Soybean phosphatides are excellent emulsifying agents and it is to this property that they owe their greatest usefulness as will become evident later. They are also presumed to possess antioxidant properties for certain fatty materials which property, however, is somewhat secondary to the property of emulsification so far as their commercial use is concerned.

The scientific, technical, and patent literature is too voluminous to review here in its entirety but the references which follow will provide a background for the general reader, and serve as a basis for further inquiry for those interested in pursuing the subject in more detail.

Much of the earlier work of importance and interest on soybean phosphatides has been carried out by German and to some extent by Japanese investigators, especially with reference to the recovery and utilization of these substances. Workers in other countries including a number in the United States have likewise made contributions to this field.

Hentze (175), Rewald (360, 354), Kühl (342), Working (471, 472, 473), Horvath (191, 190), Wiesehahn (461), and Eichberg (110) have published brief informative resumés dealing with the general aspects of the preparation, properties, and utilization of soybean phosphatides.

Much difficulty has been experienced in the United States from the separation of phosphatides from certain crude oils. In Germany where most of the soybean oil is produced by solvent extraction and then treated for the recovery of phosphatides this difficulty is not encountered. In the United States where the bulk of the crude oil is produced by pressing, the formation of sludge, especially during storage and transportation prior to refining, is often times a serious problem. Sludge formation may also occur in solvent-extracted oils if they are not subsequently treated to remove phosphatides. The phosphatides which are soluble in the crude oil at the time of pressing are gradually hydrated on standing and subsequently settle to the bottom of the tank car or storage vessel.

Jamieson and McKinney (208) reported the results of the examination of a number of American soybean oils with respect to their content of phosphatides and found that, generally, soybeans grown in North Carolina and Virginia contained less phosphatides than midwestern soybeans. The investigation of the relative content of phosphatides in various American grown soybeans was undertaken in an effort to determine the reason for the observed separation of sludge from North Carolina oils since sludge formation was presumed to be confined solely to Eastern oils. Actually, the separation of phosphatidic sludges is a rather general phenomenon (143), and is dependent on the rate and degree of their hydration. In regions or seasons of high humidity the hydration of phosphatides results in increased sludge formation and at a more rapid rate than when the climate is relatively dry. Low temperatures accelerate the rate of separation of phosphatidic sludges and once separation has occurred it is extremely difficult or impossible to completely redissolve them in the crude oil especially in storage tanks and tank cars. As has been shown by Kraybill (235), Kraybill, Kleinsmith, and Thornton (237), and Beck and Klein (30), the presence of

phosphatides in crude soybean oil is responsible for the separation of "break" material when these oils are heated to approximately 315° C.

Applications: The proposed applications for phosphatides, especially those prepared from soybean oil, are numerous indeed and new uses are constantly being suggested as even a cursory survey of the scientific and patent literature of the subject will reveal. Most of the suggested uses may be classified in the following categories: (1) food modifier, (2) cosmetics and pharmaceuticals, (3) textiles, (4) leather goods, (5) plastic compositions, (6) soaps and detergents, (7) special emulsifiers, (8) petroleum products.

(1) Food modifier: Perhaps the largest outlet for soybean phosphatides both in Europe and the United States is in the field of food modifiers in the manufacture of oleomargarine, shortening, candies and confections, bakery, milk, and other products (154, 322, 358, 360, 366). In Europe the largest use of commercial preparations of phosphatides derived from soybean oil is in the manufacture of oleomargarine, especially when made principally with coconut and palm oils (371). Phosphatide additions of 0.1 and 0.3 percent to oleomargarine reduce spattering and sticking of milk solids to the pan during frying. The dispersed water evaporates as the oleomargarine is heated and the fat froths and browns like butter. The degree of reduction of spattering during heating of phosphatide-treated oleomargarines is used in Europe as a quantitative measure of the effectiveness of commercial phosphatide preparations. This method for the comparative determination of the so-called "margarine effect" of phosphatide preparations is described in detail by Ad. Grün (385). Better moisture and salt retention in the package is likewise claimed to result from the addition of phosphatides to oleomargarine. It is also claimed (88) that by the use of phosphatides condensed and dried milk can be incorporated with fats in the preparation of oleomargarine.

The preparation and use of lecithin in vitamin compositions for use in the production of oleomargarine has been described in various patents (1, 2, 3).

Phosphatides are often added to shortenings, which according to various patent claims, result in more homogeneous mixing of the compounded fats, improved air retention, better lubricating value, and better browning qualities (113, 215, 428, 472, 473). Improvement of the characteristics of bakery goods such as pastries (276), cake fillings, icings, etc., especially with regard to texture and flavor retention are also claimed to result from the use of phosphatides in these products (214, 389).

Soybean phosphatides are used extensively in the chocolate industry in the United States as an aid in the preparation of various chocolate products, for the conservation of cocoa butter, and to improve the appearance of the finished product. The products which are benefited by incorporation of phosphatides include, especially chocolate coatings for candies, ice cream bars, fillings, caramels and other hard candies. Due to the emulsifying action and lowering of the interfacial tension by the phosphatides there occurs more complete mixing of sugar, fat (cocoa butter, butter, or shortening), water, and other ingredients (22, 213, 471). According to Avent and Morgan the addition of 1.0 percent lecithin has the most marked effect on the reduction of the

interfacial tension between cocoa butter and water.

It has been claimed that the addition of phosphatides to chocolates will result in the reduction of fat bloom or graying of the outer surface. According to Brown (67), Harris and Shillaber (164), and others, the development of bloom, which results from the diffusion of fat to the outside surface, can be prevented or retarded by the addition of 0.1 to 1.0 percent of phosphatides to the chocolate. However, other investigators, notably Clayton, Back, Robert, and Morse (85) conclude from experimental evidence that "Lecithin and lecithin substitutes, although exerting a profound influence on fluidity, exert only a slight (beneficial) effect in relation to fat bloom."

Erb and Collins (114) investigated the effect of the use of phosphatides in the application of chocolate coatings to ice cream bars. They reported that in the absence of moisture coatings containing a large amount of fat were unaffected by additions of phosphatides, but that the same coatings containing a small amount of water were markedly improved by the addition of approximately 0.4 percent of these lipids.

A process for the production of cocoa powder containing 2 percent of anhydrous phosphatides has been patented (44). According to the claims of this patent the beverage prepared from the cocoa-phosphatide powder with milk or water maintains the particles of cocoa in suspension and the cocoa butter in solution instead of separating, respectively, as a sediment on the bottom and oil droplets on the surface of the liquid.

According to another patent (87) the addition of 1.5 to 2.0 percent of commercial phosphatide-cocoa butter mixture or the fraction of soybean phosphatides insoluble in 95 percent alcohol produces a superior type of chewing gum, especially with regard to increased smoothness and to decreased tackiness and cohesiveness.

In addition to the products mentioned above the addition of phosphatides is claimed to impart beneficial or desirable properties to a host of other food products. Among these products may be mentioned sandwich spreads, peanut butter, soups, sauces, ketchup, prepared milk products, cheese, ice cream, gelatin desserts, puddings of the corn starch and chocolate type, beverages, etc. (429). Dry carbohydrate-phosphatide preparations containing various added sugars such as lactose, maltose, and malt extracts have been patented (159) as well as aqueous lecithin compositions containing high percentages of carbohydrates such as glucose or sucrose (243).

Many patents and technical articles mention, claim, or otherwise refer to the antioxidant protection which is afforded various food products by the addition of commercial soybean oil-phosphatide preparations. However, unequivocal evidence obtained under rigidly controlled and readily reproducible conditions of the antioxidant activity of such preparations is still lacking. The subject of the antioxidants which are present in soybean oil is discussed in a separate section to which reference should be made for further details.

Wolff and Guellerin (468) have investigated the effect of refining operations on the reduction of phosphatides and unsaponifiable matter in edible oils and recommend the restoration to refined olive oil of phosphatides recovered from soybean oil. Grettie (136) has patented the addition of phosphatides to winterized cottonseed oil for the purpose of inhibiting crystallization.

(2) Pharmaceuticals and cosmetics: Ad. Grün (385) discusses numerous uses of oil-free phosphatides in therapeutic preparations, the various forms in which they are dispensed, and the methods of administration in the treatment of various human and animal diseases. Besides these uses which are presumed to depend on the action in the animal organism of the phosphatides themselves or their metabolic products, other uses are made of phosphatides which depend on their emulsifying or surface tension lowering properties. Such uses include the preparation of ointments, salves, and vehicles for dispensing medicinals, tonics, vitamin preparations, liver oil emulsions, and similar products (21, 210, 354). A specially dehydrated preparation of soybean phosphatides suitable for dispensing in gelatin capsules has been patented by Buer (68).

The use of phosphatides is recommended in the preparation of facial, cleansing, and other types of creams and ointments and for emulsifying waxes, hydrocarbons, and alcohols to form oil-in-water types of prepa ons, as well as in brushless shaving creams (241), dentrifices, and similar products. Epstein and Harris (113) have patented a preparation of soybean phosphatides and diglycerides which they claim is particularly suitable for the preparation of solid lipstick having the dye dispersed in a fatty medium of high melting point and at the same time providing increased wetting capacity when applied to the tissue.

(3) Textiles: According to the claims made in the patent and technical literature phosphatides are of value in the textile and textile specialty industries because of the solubilizing effect on fatty oils, their softening power, and emulsifying properties (45, 46, 52, 60, 62, 112, 149, 150, 163, 271, 361, 363).

Mecheels (269) and Tatu (422) discuss the various applications of phosphatides in the textile industry including their use and effect in kier-boiling, as a spinning lubricant for cotton (100) and wool, addition to doubling solutions, in the mercerizing bath as a wetting-out agent, in the dye bath in rayon finishing as a leveling agent, and for increasing color in calico printing pastes (157). Bollmann and Rewald (61) and others (153) have patented the use of phosphatides in dyeing cotton, wool, silk, rayon, and other textile materials and claim certain advantages such as increased brilliance, softer handle, and better penetration.

Ohl (326) discusses the use of phosphatides as a stabilizing agent in bleaching rayon and other synthetic yarns. In Europe the use of phosphatides as a wetting and softening agent and in the preparation of sizing and finishing pastes has been patented (52, 53, 192, 342). Bollmann and Rewald (51) and Rewald (364) have patented the use of phosphatides in printing textile fabrics and claim many advantages are to be derived therefrom.

The production and use of hydrated (442,255) and sulfonated (49, 77, 78) phosphatides has been covered in a series of patents.

(4) Leather: In recent years the use of phosphatides has been advocated or introduced as an assistant in producing oil- and chrome-tanned leathers (32, 39, 76). They are recommended for use in fat-liquoring to facilitate the penetration of fats and oils into the hide, to reduce the amount of working required, and to make the finished leather more pliable (353). The use of soybean phosphatides in tanning preparations, softening oils, leather dressings, and greases is described in a considerable number of patents which should be consulted for details of their application (47, 54, 55, 56, 57, 58, 59, 161, 162, 341, 357).

(5) Plastic compositions: The use of soybean phosphatides has been recommended for the production of various plastic compositions including vulcanized rubber (115, 362), linoleum, and sound records (123). According to claims in the literature phosphatides are especially useful in the production of putty, casein paints, in the vulcanization of rubber, for reducing foaming in latex, and decreasing the rate of ageing in linoleum.

Merz and Wagner (272) and Stock (406) have discussed the application of phosphatides in the manufacture of oil paints and casein water paints. The use of phosphatides as a binder in paints has been patented by Bollmann and Rewald (50), Fincke (160), and others.

The New York Paint and Varnish Production Club (315, 316) has reported studies on the wetting and dispersion of pigments in oils containing added phosphatides. The production of asphalt and tar emulsions in which soybean phosphatides are employed as emulsifying agents has been patented both in the United States (387) and abroad (152, 158).

(6) Soaps and detergents: The effect of phosphatides on the composition and physical properties of soaps has been discussed by Lederer (246), Genin (128), and others. Inaba, Kitagawa, and Sato (194) observed that the addition of lecithin to soap solutions resulted in a lowering of the surface tension as measured by the drop number against kerosene. It was also found to affect the foam number, turbidity and transparency temperatures, and the gold numbers. Trusler (434) investigated the effect of the presence of phosphatides on the rate of catalytic splitting of fats and found the reaction to be inhibited by these substances. However, after treatment of the fat with dilute mineral acids, as for example with one to three percent hydrochloric acid, it was found catalytic hydrolysis could be effected.

The manufacture of soap containing phosphatides has been patented (155) and soaps of this type are said to have better foaming properties, especially when they contain excessive amounts of sterols. The presence of phosphatides serves as an aid in superfatting and in general to impart improved texture to fine toilet soaps. Braun and Rosenbusch (66) have patented a liquid preparation consisting of phosphatides, castor oil fatty acids, and poly-ethanolamines for use in the production of soaps.

(7) Special emulsifiers: Since the primary action exerted by phosphatides in aqueous and other solutions is one of lowering surface tension and increasing emulsifying action it is not surprising that numerous applications in this field have been investigated and patented. In addition to those already cited in connection with various food, cosmetic, and textile preparations, many others might be cited particularly with reference to certain special applications.

The use of phosphatides in the production of adhesives has been described (148) and the addition of phosphatides to insecticide and fungicide preparations containing mineral oil and water-soluble substances, e.g., copper salts, has been patented (48, 359). Bousquet (64) has patented the use of crude soybean phosphatides in contact sprays containing nicotine, rotenone and pyrethrum. According to the claims of the patentees the presence of phosphatides results in better dispersion of the phases and greater adhesion of the applied spray. However, Woodman (469) on the basis of extensive studies of numerous polyphase systems containing lecithin concluded, "An aqueous dispersion of lecithin has been demonstrated to form dual emulsions with certain non-aqueous liquids, the water-in-oil type only with oleic acid, and oil-in-water only with certain bases. Generally speaking, therefore, lecithin is an unsuitable emulsifier for the preparation of insecticidal and ovicidal spraying emulsions."

According to a patent of Bollmann and Rewald (62) emulsions of phosphatides with or without added oils can be prepared solely with such solvents as benzyl alcohol, ethylene, propylene and amylene glycols, and many aromatic alcohols. Similar preparations have been patented by others (151).

An alkali peroxide-stabilized emulsion consisting of crude undried phosphatide-soybean oil mixture has been patented by Engelmann (112). Dry, powdered preparations of phosphatides with lime and with solid sodium hydroxide have been patented, respectively, by Bollmann (43) and by Schwieger (387).

(8) Petroleum products: It is claimed in patents by Bollmann (402) and others (156) that the addition of phosphatides to gasoline will prevent or at least retard the formation of gums. Hendrey (174) has patented the use of phosphatides in lubricating oils and claims that the addition of 0.1 to 1.0 percent of phosphatides to such oils has been found to reduce their ring-sticking behavior when used in lubricating automotive engines. Musher (299) claims that the combination of commercial soybean lecithin and a polyhydroxy compound decreases sludge formation in lubricating oils.

White and Vaughan (460) found that the addition of 0.25 to 2.0 percent of phosphatides to wood-preserving oils, such as creosote and mineral oil, increased the penetration and improved the distribution of the preservative in the wood.

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